THE BIO-ECOLOGY OF THE CAPE GRAPEVINE LEAFMINER, *HOLOCACISTA CAPENSIS* (LEPIDOPTERA: HELIOZELIDAE), IN THE WESTERN CAPE

by

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Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

March 2016

Abstract

The Cape grapevine leafminer, *Holocacista capensis* Nieukerken & Geertsema (Lepidoptera: Heliozelidae), occurring on *Vitis vinifera* L., has recently become of economic importance in the Western Cape, South Africa. The leafminer is of quarantine importance as cocoons are often attached to grape bunches intended for export when grapevine infestation is severe.

Fortnightly monitoring efforts have indicated the occurrence of several overlapping generations within a growing season which are likely to affect potential management strategies. Adult and larval abundances tend to increase as the grapevine growing season progresses and as temperatures increase. A peak in adult and live larval abundance is reached between February and March, usually after harvest. The severity of leafminer infestation may be affected by the location of a vine within a cultivated block and strong evidence suggests that structural aspects (i.e. the trellis system used) affect the invasion potential of *H. capensis*.

The leafminer tends to overwinter in the larval or pupal life stage within a cocoon which is often sheltered under the bark on the stem of a grapevine, in leaf litter or in a small crevice of a trellis post. Male moths have been detected throughout the colder winter months suggesting that small fluctuations may affect the developmental rate of overwintering pupae depending on the shelter experienced by cocoons. It is a possibility that adults produce offspring in surrounding foliage or are sourced by such refuges, although it is likely that eclosed adults undergo diapause and contribute to the first generation of leafmining larvae as soon as conditions become favourable.

Pheromone baited traps, placed throughout the Western Cape, have indicated the presence of the moth in two of the largest table grape producing regions, namely the Berg River and Hex River Valley. The overall distribution of the moth has proven to be far more extensive than previously thought and may be present throughout southern Africa based on herbarium samples that yielded symptoms of leafminer damage.

Monitoring strategies should be conducted as early as possible within a grapevine growing season to avoid severe infestations and unnecessary chemical applications depending on the bunch infestation tolerance. At an economic threshold of 5% bunch infestation, the commencement of management strategies is recommended when 442 adult male moths are caught per trap per fortnight. Results suggest that bunch infestation could be prevented altogether if management strategies were implemented when less than 87 adult male moths are caught.

In cases where bunch infestation has occurred and post-harvest cold treatments are considered, a cold-treatment exposure of no less than 26 days at -0.5°C should be considered if 100% mortality is desired.

Parasitoid wasps associated with the leafminer have been identified and could potentially be used in the area-wide control of *H. capensis*. A variety of chemical compounds have been used to control leafminer infestations in the past, although preventative applications of spinetoram seem to have had the most success in preventing infestation. Management strategies in line with the ideals of current Integrated Pest Management strategies should be explored in future research endeavours.

Opsomming

Die inheemse Kaapse wingerdblaarmyner, *Holocacista capensis* Nieukerken & Geertsema 2015 (Lepidoptera: Heliozelidae), wat op *Vitis vinifera* L. voorkom het onlangs van ekonomiese belang geraak in die Wes-Kaap, Suid-Afrika. Die blaarmyner se kwarantyn is van beduidende belang as ŉ potensiële kwarantynplaag omdat kokonne dikwels aan druiwetrosse kleef wat bestem is vir uitvoer, veral onder swaar bestemmings.

Twee-weeklikse monitering het aangedui dat daar verskeie oorvleuelende generasies binne 'n enkele seisoen voorkom wat waarskynlik potensiële bestuurstrategieë beïnvloed. Volwassenes en larwe verspreidings neig om toe te neem soos die wingerdstok se groeiseisoen vorder en met toenemende temperature. 'n Hoogtepunt in die volwasse en lewende larwe getalle word bereik tussen Februarie en Maart, gewoonlik na oes. Die omvang van blaarmynerbesmetting mag beïnvloed word deur die posisie van die wingerdstok binne 'n bewerkte blok en sterk bewyse dui daarop dat strukturele aspekte (d.w.s die tipe traliewerkstelsel wat gebruik word) die inval potensiaal van *H. capensis* binne 'n besmette area grootliks beïnvloed.

Die blaarmyner neig om te oorwinter in die larwe- of papie stadium binne die kokon wat dikwels beskut is onder die bas op die stam van 'n wingerdstok, in blaarmolm of in 'n klein kraak van die traliewerk. Manlike motte is tydens kouer wintermaande gevind wat daarop dui dat klein fluktuasies die ontwikkelingsbehoeftes en koers van oorwinterende papies beïnvloed, afhangende van die graad van beskutting wat motte ervaar. Dit is moontlik dat volwassenes nageslag produseer in die omliggende blare, of aangevul word deur sulke toevlugsoorde alhoewel dit hoogs waarskynlik dat nuwe volwassenes diapause ondergaan en so tot die eerste generasie van blaarmyner larwes bydra sodra toestande gunstig raak.

Feromoon-aas lokvalle wat oor die hele Wes-Kaap geplaas is, het aangedui dat die mot in twee van die grootste tafeldruifstreke, naamlik die Bergrivier en Hexrivier Vallei, teenwoordig is. Oor die algemeen is die algehele verspreiding van die mot veel meer uitgebrei as voorheen gedink en mag die mot dwarsdeur Suider-Afrika teenwordig wees, gebaseer op herbarium monsters wat blaarmyner skadesimptome vertoon.

Moniteerstrategieë moet so vroeg as moontlik gedoen word binne 'n wingerdstok groeiseisoen om ernstige besmettings en onnodige voorkomende chemiese toedienings, afhangende van die tros besmetting verdraagsaamheid, te vermy. By 'n ekonomiese drempelwaarde van 5% tros besmetting, word bestuurstrategieë aanbeveel wanneer 442 volwasse manlike motte gevang word per lokval oor twee weke. Resultate dui daarop aan dat as bestuurstrategieë geïmplementeer word wanneer daar minder as 87 volwasse manlike motte versamel is, kan tros besmetting heeltemal voorkom word.

In gevalle waar tros besmetting plaasgevind het en na-oes koue behandeling oorweeg word, moet die koue behandeling blootstelling ten minste 26 dae lank wees teen -0,5°C indien 100% mortaliteit verlang word.

Parasitiese wespe geassosieerd met die blaarmyner is geïdentifiseer en kan moontlik gebruik word in die gebied-wye beheer van *H. capensis*. 'n Verskeidenheid van chemiese middels is in die verlede gebruik is om blaarmynerbesmettings te beheer, alhoewel voorkomende programme met spinetoram blyk asof dit mees suksesvol is vir die voorkoming van blaarmynerbesmetting. Bestuurstrategieë in pas met die ideale van die huidige Geïntegreerde Plaagbestuur strategieë moet in toekomstige navorsing pogings ondersoek word.

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Chapter 1

Literature review

Of all insects, only four insect orders are known to contain leafmining species (Hering 1951). The Lepidoptera and Diptera have the largest number of leafmining species whilst those of the Coleoptera and Hymenoptera are relatively scarce. The mines originating from these four orders are classified into specific groups, namely: lepidopteronome, dipteronome, coleopteronome and hymenopteronome, respectively (Hering 1951). Due to the destructive qualities of some leafmining species the Lepidoptera are considered to be of significant economic importance (Nielsen & Common 1991).

Leafmining insects are, in essence, phytophagous with their larvae concealed in plant tissue (Hering 1951). Most of the current knowledge on leafminers is due to research carried out in the European and American regions, although relatively little is known of leafmining insects globally (Vári 1961; Auerbach *et al*. 1995; Lees *et al*. 2014).

1.1. Systematics

Despite the fact that Lepidoptera are the most recently evolved major insect order, their radiation was rapid as the fundamental clades of Lepidoptera were all represented before the beginning of the Tertiary and paralleled that of the angiosperms which became prominent between ca. 140 and 90 mya (Powell 2003; Wahlberg *et al*. 2013).

The Early Cretaceous yielded the second oldest group of Lepidoptera, known as a basal grade of mandibulate moths, which include Micropterigidae, Agathiphagidae and Heterobathmiidae (Grimaldi & Engel 2005). Heterobathmiidae is the first lineage to contain leafmining larvae. The Glossata, a large monophyletic group comprising of all other moths are the "tongued" moths. The Neolepidoptera is a major lineage within the Glossata and contains the non-ditrysian and ditrysian Heteroneura. The moths within the Early Heteroneura include small, diurnal moths with heteroneurous wing venation, frenular wing coupling and associated increase in size difference between the pterothoracic segments (Holloway *et al*. 1987; Scoble

1992; Regier *et al*. 2015). It was considered that the minimum age of the Heteroneura was 120-125 Mya due to the discovery of a well preserved, undescribed, adeloid fossil found in Lebanese amber of the Barremian or Aptian age (in the early Cretaceous) (Labandeira *et al*. 1994). More recent studies by Sohn *et al*. (2012) and Wahlberg *et al*. (2013) have reported on the age and origin of several other Heteroneuran fossils, the earliest of which date back to the Kimmeridgian age of the Jurassic period. The minimum age of the Heteroneura is, therefore, 152-157 Mya. A defining character of the Ditrysia is the presence of a significant morphological structure in females: an internal passage through which sperm can pass between the copulatory tract and the oviduct (Grimaldi & Engel 2005).

The Monotrysian or nonditrysian superfamilies represent less than one percent of known extant Lepidoptera and are described as minute to small day-flying microlepidoptera with monotrysian female genitalia (Holloway *et al*. 1987; Scoble 1992), a trait which is considered to be plesiomorphic (Holloway *et al*. 1987). Moths possess a frenulum-retinaculum wing coupling mechanism and have lost the first abdominal sternite (Bourgogne 1951; Powell 2003).

The six most basal superfamilies characterized within the Early Heteroneura (Andesianoidea, Adeloidea, Nepticuloidea, Palaephatoidea and Tischerioidea) do not, collectively, form a monophyletic group as they share no derived characters (Scoble 1992; Powell 2003) as they retain the ancestral monotrysian female reproductive system (Powell 2003).

Davis (1998) suggests that the presence of spine combs on male valva (pectinifers) and metafurcasternum (with two pairs of dorsal tendons and without secondary arm lamellae) are potentially two synapomorphies for the monophyly of the monotrysian Heteroneura. Nepticulidea, Adeloidea and Tischerioidea are the most diverse of the superfamilies (Powell 2003; Regier *et al*. 2015).

A key to the Monotrysian superfamilies and families is as follows (adapted from Davis 1998):

1. Female with elongate piercing ovipositor (Fig. 1.1, G – K). Male genitalia usually with elongate, sagittate, or deeply furcate juxta (Fig. 1.1; C, F, Adeloidea)…….....2
- Ovipositor short, non-piercing. Juxta absent or variably shaped, never sagittate nor deeply furcate..…7
2. Hindwing with discal cell open (M-CuA crossvein absent (Fig. 1.2, A – B).……*Heliozelidae*
- Hindwing with discal cell closed……...3
3. Antennae usually longer than forewing……*Adelidae*
- Antennae shorter than forewing……...……4
4. Female with apex of ovipositor depressed (Fig. 1.1, H – I). Male with juxta not sagittate, either broadly angulate or deeply furcate. Mature larva casebearing…..…5
- Ovipositor compressed (Fig. 1.1, K). Male juxta usually slender, sagittate. Larvae endophagous, never casebearing……..……6
5. Male pectinifer consisting of a single large spine. Larvae leaving case to mine host leaf; A3-6 with biserial crochets…….....*Incurvariidae*
- Male pectinifer multispined or absent. Late instar larvae usually exophytic; A3-6 with uniserial crochets……..……*Incurvariidae*
6. Adult with haustellum usually reduced, less than first labial palpal segment in length, or absent; maxillary palpy short, 3-segmented to absent. Frenulum-retinaculum absent. Larvae cecidogenic…..…
……..*Cecidosidae*
- Adult with haustellum developed; maxillary palpi elongate, 4-5 segmented. Frenulum-retinaculum present. Larvae usually boring in fruits, leaves or stems, rarely cecidogenic..……*Prodoxidae*
7. Discal cell open in forewing; antennae scape usually greatly broadened, covering eye (Nepticuloidea, Fig. 1.3, J – K)...……..……..……8
- Discal cell closed in forewing; scape unmodified, never forming eyecap……...…..9
8. Forewing with branched or stalked, often arcuate veins (Fig. 1.2, I)..……*Nepticulidae*
- Forewing with all veins separate and relatively straight (Fig. 1.2, J)..……....*Opostegidae*
9. Hindwings slender; width 0.23 the length or less; third antennal segment 2.5-3X length of first (Fig. 1.3, M, Tischerioidea)…….……...…….……*Tischeriidae*
- Hindwings broader, width 0.33 the length or more; third antennal segment less than length of first (Palaephatoidea)……..…....*Palaephatidae*

The most recent study focused on establishing a more accurate depiction of the molecular phylogeny of the nonditrysian lineages was by Regier *et al*. (2015).

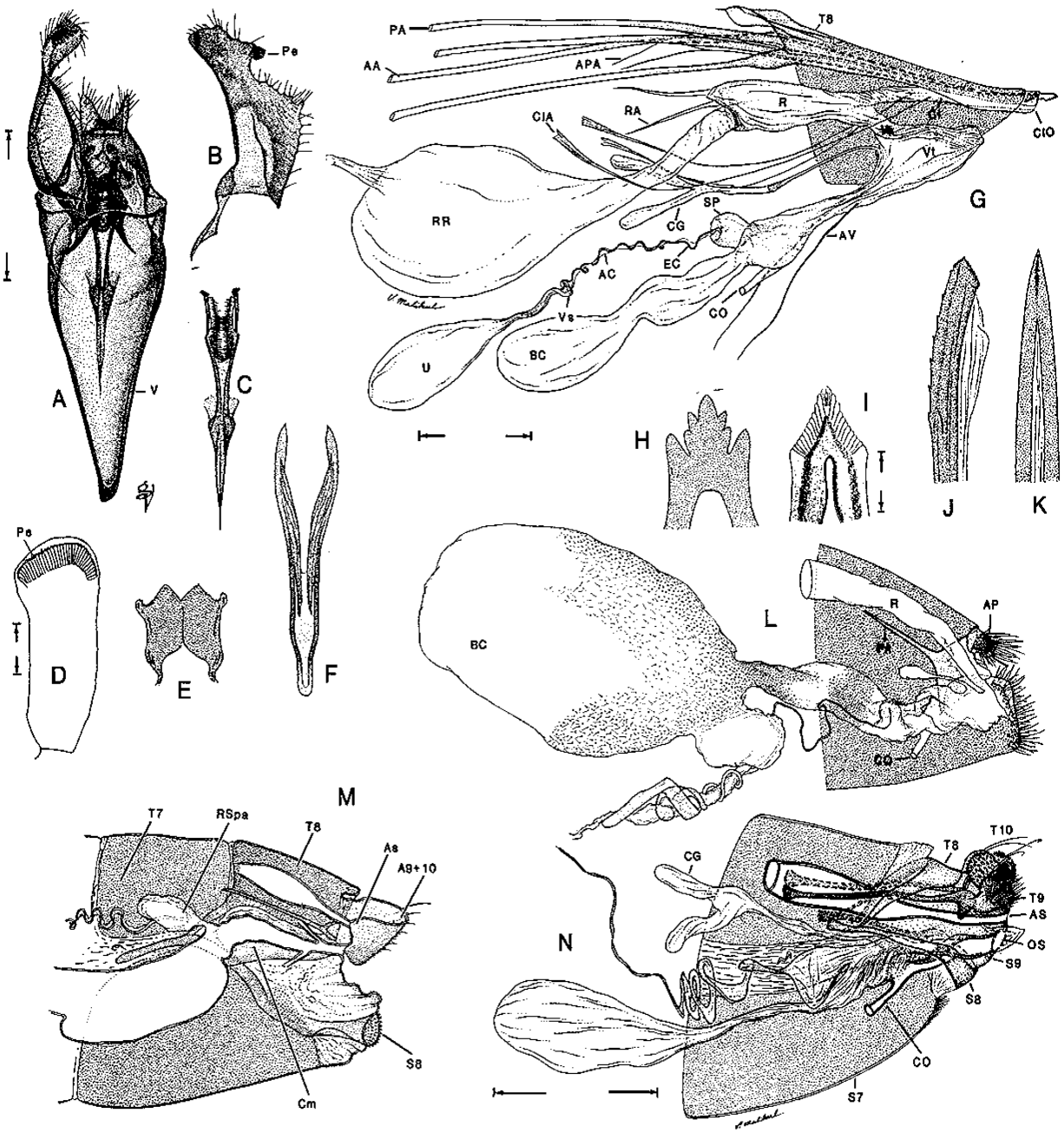

Figure 1.1: Genital morphology. A-F, male genitalia: A-C, Prodoxidae, *Lampronia capitella*: A, ventral view; B, Valva, mesal view; C, juxta, ventral view; D, Nepticlidae: *Pectinivalva commoni*, male valva; E, Crinopterygidae: *Crinopteryx familiella*, male juxta; F, Incurvariidae: *Incurvaria vetulella*, male juxta; G-N, female genitalia: G, Adelidae: *Ceromitia fasciolata*, lateral view; H-K, apices of ovipositor: H, Crinopterygidae: *Crinopteryx familiella*, ventral view; I, Incurvariidae: *Perthida glyphopa*, ventral view; J-K, Prodoxidae: *Prodoxus quinquepunctellus*: J, lateral view; K, ventral view; L, Opostegidae: *Pseudopostega bistrigulella*, lateral view; M, Palaephatidae: *Palaephatus falsus*, lateral view; N, Tischeriidae: *Tischeria citrinipennella*, lateral view (AA = anterior apophysis, AC = afferent (transport) canal, AP = anal papilla (A10), APA = apodeme of posterior apophysis, As = anus, AV = apodeme of vestibulum, BC = bursa copulatrix, CG = colleterial gland, Cl = cloaca, ClA = cloacal apodeme, ClO = cloacal opening, Cm = colliculum, CO = common oviduct, EC = efferent (fertilization) canal, Os = oviporus, PA = posterior apophysis, Pe = pectinifer, R = rectum, RA = rectal apodeme, RR = rectal reservoir, S = sternum, SP = spermathecal papilla, T = tergum, U = utriculus of spermatheca, V = vinculum, Va = vagina, Vs = vesicle, Vt = vestibulum). All scales = 0.5 mm. Taken from Davis (1998).

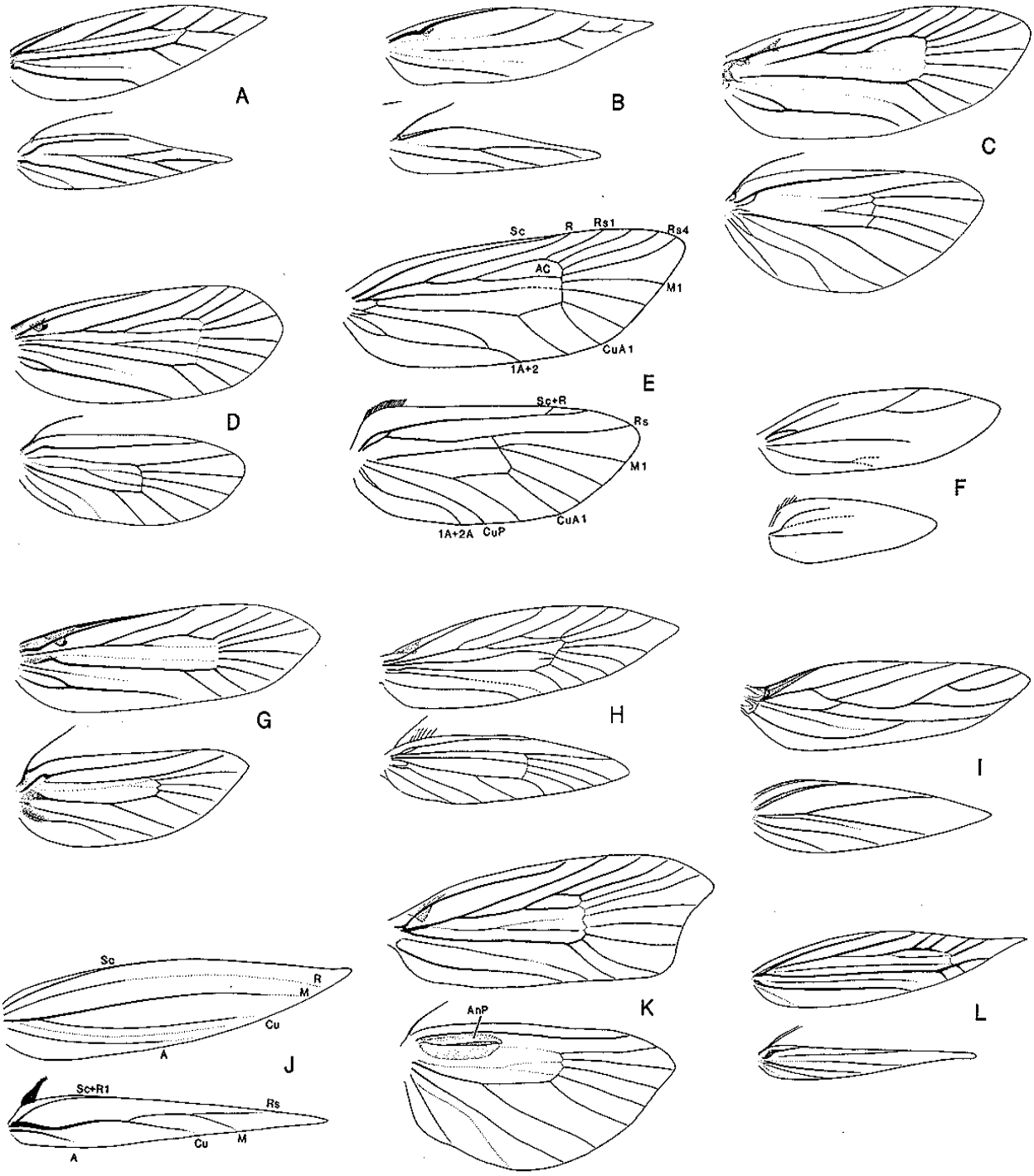

Figure 1.2: Wing venation. A - B, Heliozelidae: A, *Heliozela aesella*; B, *Coptodisca splendoriferella*; C, Adelidae: *Adela trigrapha*; D, Prodoxidae: *Lampronia luzella*; E - F, Cecidosidae: E, *Cecidoses eremita* (AC = accessory cell); F, *Dicranoses congregatella*; G, Incurvariidae: *Incurvaria masculella*; H, Crinopterygidae: *Crinopteryx familiella*; I, Nepticulidae: *Ectoedemia phleophaga*; J, Opostegidae: *Opostega salaciella*; K, *Palaephatus falsus* (AnP = androconial pocket); L, Tischeridae: *Tischeria quercitella*. Taken from Davis (1998).

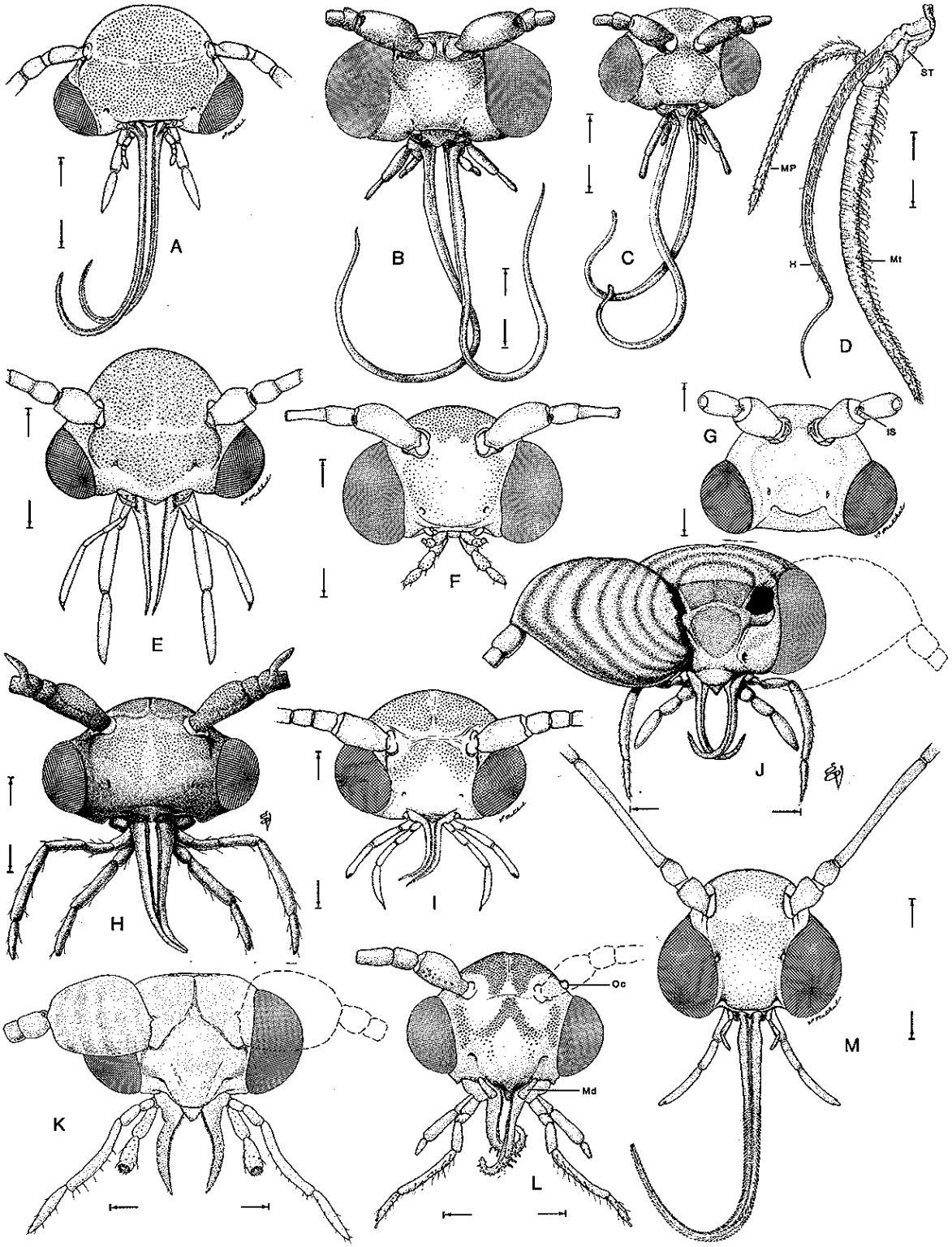

Figure 1.3: Head morphology. A, Heliozelidae: *Heliozela aesalla*; B - C, Adelidae, *Adela reaumurella*: B, male; C, female; D - E, Prodoxidae: D, *Tegeticula yuccasella*, female maxilla (H = haustellum; MP = maxillary palpus; MT = maxillary tentacle; ST = stipes); E, *Lampronia luzella*; F – G, Cecidosidae: F, *Oliera argentinana*; G, *Dicranoses congregatella*, (IS = intercalary sclerite); H, Incurvariidae: *Incurvaria masculella*; I, Crinopterygidae: *Crinopteryx familiellla*; J, Opostegidae: *Pseudopostega bistrigulella*; K, Nepticulidae: *Ectoedemia phleophaga*; Lm Palaephatidae: *Palaephatus falsus*, (Md = mandible, Oc = ocellus); M, Tischeriidae: *Tischeria zelleriella*. All scales = 0.5 mm. Taken from Davis (1998).

1.2. Heliozelidae

Moth species within the superfamiliy Adeloidea [formerly described as Incurvarioidea (Nieukerken *et al*. 2011)] are usually fairly drab but several are metallic (Scoble 1992; Regier *et al*. 2015). Biologies and larval habits vary amongst species, however, all construct portable cases at least in the last larval instar (Scoble 1992; Powell 2003). Many species are exophagous and feed on detritus, although endophagy is widespread within the group (Scoble 1992). The Adeloidea contains six diverse families: Heliozelidae, Adelidae, Crinopterygidae (larval case bearers), Incurvariidae, Cecidosidae (gall inducers) and Prodoxidae (Holloway *et al*. 1987).

The Heliozelidae (Lepidoptera, Adeloidea) is a group of widely distributed monotrysian microlepidoptera occurring in all major faunal realms except New Zealand and Antarctica (Davis 1998; Powell 2003). Heliozelids are most diverse in North America and Australia (Nieukerken *et al*. 2012), and have been reported throughout Europe (including Finland, Netherlands, Italy and France), Asia (including India, Vietnam, Indonesia, Malaysia, Taiwan and Japan) and South Africa (Nielsen & Common 1991; Scoble 1992; Nieukerken *et al*. 2012). Species of this family are rarely seen due to their minute size and diurnal habit, although many more species are known from their characteristic larval mines (often abandoned when found) (Powell 2003). The family is, taxonomically, poorly studied and comprises 125 described species in 12 genera (Nieukerken & Geertsema 2015).

A key to the genera of Heliozelidae (adapted from Mey 2011):

1. Forewing with discoidal cell……..2
- Forewing without discoidal cell, R1 present as isolated vein……..*Antispilina* Hering, 1941
2. Discoidal cell until middle of forewing, metallic fascia present……*Antispila* Hübner, 1825
- Discoidal cell longer, beyond middle of forewing, fascia absent, with 1-2 dorsal spots…….……
  ……*Heliozela* Herrich-Schäffer, 1853

1.3. Morphology

In most cases, heliozelid moths possess fundamentally dark ground colouration with iridescent, metallic-appearing scales (Fig 1.4) (Scoble 1992; Powell 2003). Two of the three specialized characteristics for Heliozelidae are cephalic: 1) the anterior tentorial arms are curved dorsally, the vestigial maxillary palpi are significantly reduced (rarely 5-segmented) and the scales on the vertex of the rough-scaled head are appressed to the cranium; 2) the antennae are fairly short and 3) the narrower hindwing is characterized by the absence of the M-CuA crossvein and the venation of lanceolate wings is strongly reduced (reduced radial system) (Bourgogne 1951; Holloway *et al*. 1987; Powell 2003).

Davis (1998), among others, describe the general morphology of the Heliozelidae as follows:

Head

Heads are typically smooth and covered with broad, laminate, iridescent scales directed downwards over smooth frons (Fig. 1.5, A) (Davis 1998). The vertex of the head of one undescribed South American genus is typically rough with piliform scales (Davis 1998). Similar to other diurnal microlepidoptera, adults possess small eyes (Davis 1998; Powell 2003). The cornea possesses sparse, scattered interfacetal microsetae (Davis 1998). The antennae are approximately 0.6 – 0.7 the length of the forewing; possess a smooth scape (covered entirely with usually iridescent scales); absent of pectinifer and possess a piliform flagellum (fully scaled to apex but more sparsely scaled ventrally).

The unscaled haustellum is moderate to long (1.5 – 2.0 the length of labial palpi) and in some instances, the base is concealed by elongate, lamellar scales from the labrum where pilifers may or may not be present (Davis 1998). The maxillary palpi (reduced predominantly in length) are not geniculate and are usually comprised of 1 – 5 short segments (Davis 1998; Nieukerken & Geertsema 2015). The moderately short labial palpi are typically 3-segmented and drooping (Davis 1998).

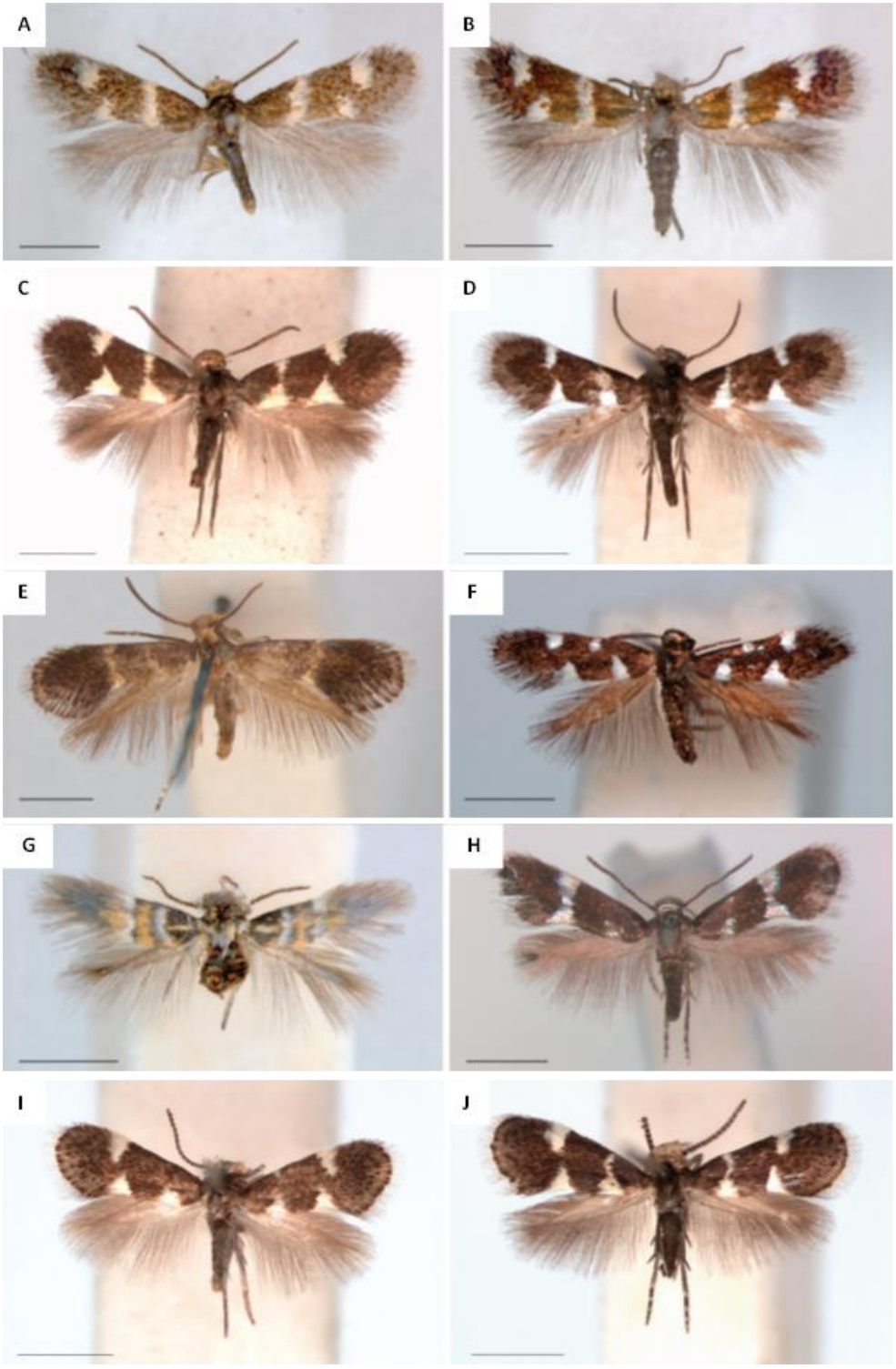

Figure 1.4: *Holocacista* species, adult habitus. *H. varii* , male (A) and female (B); unidentified heliozelid species, males (C, D, F, G, H); *H. selastis*, male (E); *H. capensis*, male (I) and female (J). All scales = 1 mm. Adapted from Nieukerken & Geertsema (2015).

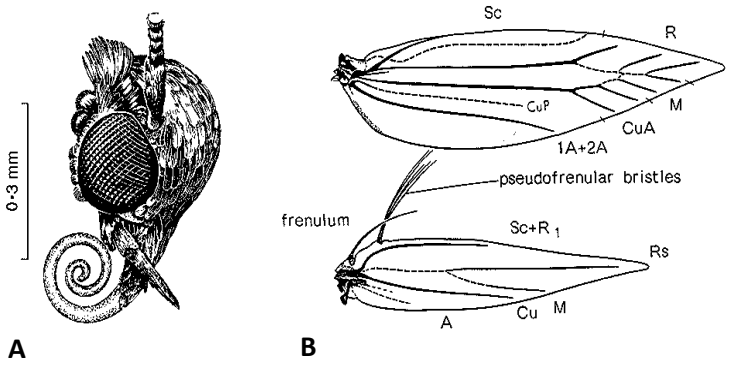

Figure 1.5: The head (A) and wing venation (B) of *Heliozela*, Heliozelidae. Adapted from Nielsen & Common (1991).

Thorax

The lateral angles of laterocervical sclerites are broad and triangular (Fig. 1.6, A) (Davis 1998). The metafurca (Fig. 1.6, B) possesses moderate- to well-developed dorsal apophyses which arise perpendicularly from the mesal lamella (free from secondary arms of metafurcasternum). The legs of adults have a tibial spur pattern of 0-2-4 (Davis 1998).

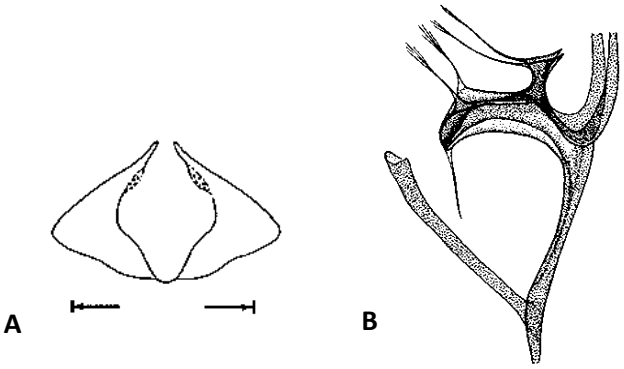

Figure 1.6: Thoracic morphology. The lateral cervical sclerites (A) and metathoracic furcasterna, lateral view (B) of Heliozelidae, *Heliozela aesella*. All scales = 0.5 mm. Adapted from Davis (1998).

Wings

Forewings (Fig. 1.2, A – B), characterized by silver spots or fasciae on the forewings (Scoble 1992), are broadly lanceolate, index 3.4 – 3.6; microtrichia are mostly restricted to the subhumeral and subanal areas; scales are often lustrous; radius with three or four branches; Rs4 stalked to M1 and terminating on costa near apex; discal cell either open or closed; accessory cell absent; M with one or two branches; CuA with one or two branches; 1A + 2A

without basal fork; in males, retinaculum is a triangular lobe from the costa; in females, retinaculum consists of three to four stout, curved spines from a small swelling at the base of Sc (Davis 1998). The principal autapomorphy for the Heliozelidae is the loss of the M-CuA crossvein in the hindwing (Davis 1998).

Hindwings are more slender, lanceolate, index 0.2 – 0.28; M-CuA crossvein absent, discal cell open; M with one or two branches; 1A + 2A are usually fused, rarely divided near margin; frenulum a single large bristle in male with four to six smaller costal setae and with usually two small bristles in females (Davis 1998).

Heliozelids are of the smallest moths known with heteroneurous wing venation (Holloway *et al*. 1987; Scoble 1992) and the presence of greatly reduced or dissimilar fore- and hindwing venation is considered to be an apomorphic trait (Fig. 1.5, B) (Holloway *et al*. 1987).

Abdomen

The anterior third of S2 is characterized by a broad U-shaped caudal rim (Fig. 1.7, A) and the S7 caudal rim of females is rounded (Fig. 1.7, B) (Davis 1998).

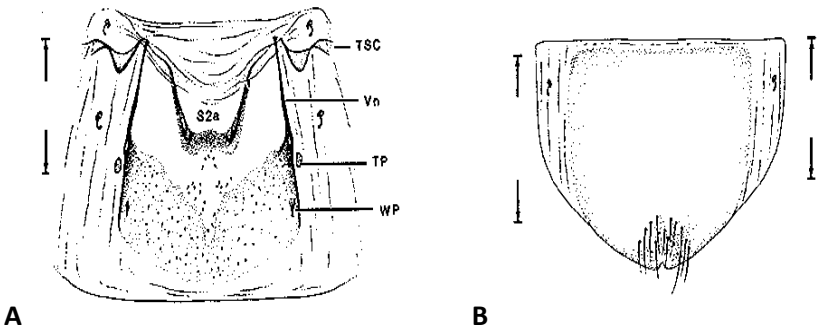

Figure 1.7: Abdominal morphology. Sterna 2 (S2) (A) and 7 (S7) (B) of Heliozelidae, *Heliozela aesella.* All scales = 0.5 mm. Adapted from Davis (1998).

Male genitalia

The vinculum is well developed with an elongate, broad, V-shaped saccus (Davis 1998). The uncus is indistinct and shallowly bilobed. Valvae are characterized by a single pair of pectinifers, each bearing approximately five to twenty spines. Juxta are usually slender,

sagittate. The phallus is an elongate tube usually equalling the length of saccus. Cornuti are present (Davis 1998).

Female genitalia

The depressed ovipositor terminates in four to five minute cusps and has a moderately broad apex (Davis 1998). The corpus bursae (without signa) is entirely membranous and, in some cases, the ductus bursae is lined with small spines (Davis 1998).

Eggs

The eggs of heliozelids are poorly known, although most females insert eggs singly beneath the bark of a twig of a hostplant or directly into a leaf or petiole where hatched larvae feed for a time in the midrib producing a characteristic "leaf mine" (Scoble 1992; Davis 1998). Where larvae hatch in the bark, larvae mine from the twig into the petiole of the leaf and proceed to the lamina (Scoble 1992).

Larvae

The white to pale yellow or green larvae are approximately four to six millimetres in length and are flattened, usually legless leafminers when fully grown (Fig. 1.8, A – B) (Scoble 1992; Davis 1998; Powell 2003). The pronotum and anal plate may be brownish or black and, in some cases, a series of nearly contiguous, mid-dorsal dark plates run down the length of the body (Davis 1998). The head is strongly depressed and mostly prognathous with two to five pairs of stemmata (Davis 1998). In addition the heads of larvae are usually dark and possess strongly chitinised head capsules, characteristics which distinguish Lepidoptera from the leafmining larvae of the Diptera (Hering 1951; Davis 1998).

The thoracic legs and abdominal prolegs of larvae are, in most cases, absent or reduced to fleshy protuberances (Holloway *et al*. 1987; Scoble 1992; Davis 1998). When present, thoracic legs are well developed, 5-segmented (seen in some *Heliozela*) or represented by paired ambulatory calli situated dorsally and ventrally on the thorax (T1 and 2) (Scoble 1992; Davis 1998). If present, coxal plates are separated (Davis 1998). Crochets are usually absent, if present (Fig. 1.8, C) they are typically arranged in multiserial rows consisting of enlarged spines (resembling those of Adelidae) on A3 - 6 (Scoble 1992; Davis 1998). Crochets are always absent on A10 (Davis 1998). In the case of some species of *Antispila*

and *Coptodisca* however, ventral ambulatory calli on A3 - 6 are fused (Scoble 1992; Davis 1998).

The final instar constructs a flat, oval case by cutting sections from the upper and lower epidermal layers of the leafmine produced by a feeding larvae (Davis 1998). Pupation will occur within the lenticular casings once settled. The common names "shield bearers" and "leafminers" are derived from larval habits and constructed pupal casings (Scoble 1992; Davis 1998).

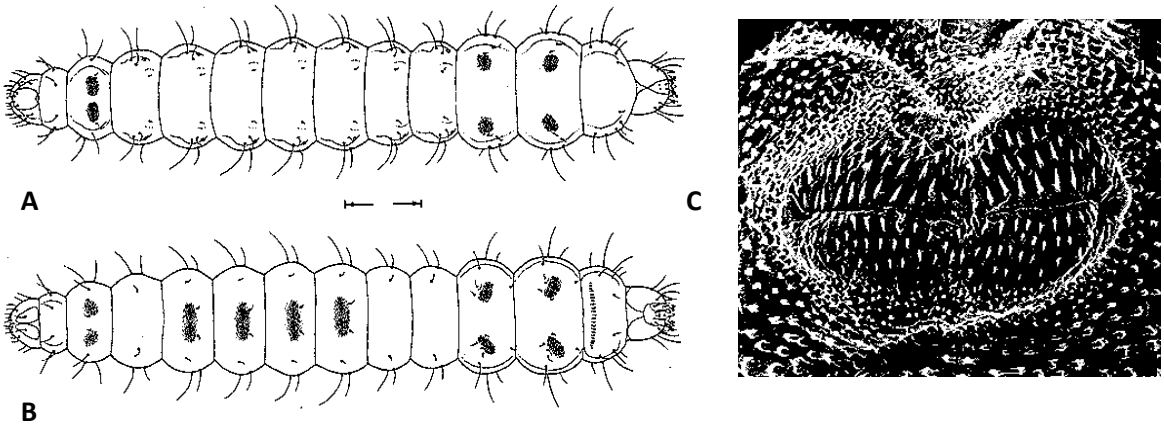

Figure 1.8: Immature stages (A – B) and larval crochets (C) of Heliozelidae. The dorsal (A) and ventral (B) view of the larvae of *Coptodisca arbutiella*. A3 (108 µm) larval crochet (C) of *Heliozela aesella*. All scales = 0.5 mm. Adapted from Davis (1998).

Pupae

Pupae within the monotrysian Heteroneura are typically adecticous-obtect and possess free abdominal segments (Holloway *et al*. 1987). Pupae are semiliberae and ca. 1-4 mm in length (Patočka & Turčáni 2005). The cuticle is thin and transparent (Davis 1998); the sculpture is smooth and setae are visible (Patočka & Turčáni 2005). Their heads are smoothly rounded; the vertex is relatively long; antennae are short (less than half the length of the forewing) and the scape is cylindrical (Davis 1998; Patočka & Turčáni 2005). Labial palpi on the labium are distinct and the maxillae are elongated into a short or medium short proboscis (Patočka & Turčáni 2005). The maxillary palpi are conspicuous (Patočka & Turčáni 2005). The forewings are pointed and extend to the sixth or seventh abdominal segment (Fig. 1.9; A, C,

E) (Patočka & Turčáni 2005). The pronotum is short and the metanotum is deeply concaved. Visible hindwings are short (Patočka & Turčáni 2005).

Dorsally, the abdomen lacks larger spines or spine stripes, however, it is covered in dense patches of minute, scattered spines (Davis 1998; Patočka & Turčáni 2005). In males, segments A2 – 8 are moveable whereas in females, only segments A2 – 7 are moveable. Spiracles may either be raised or flush (Davis 1998). The end of the abdomen is obtuse and a cremaster is not present (Fig. 1.9; A, C, E) (Patočka & Turčáni 2005). Their body shape is described as fusiform and of medium slenderness (Patočka & Turčáni 2005).

Patočka & Turčáni (2005) developed keys to the genera and species within the Heliozelidae.

Key to genera:

1. Antennae not or barely longer than prothoracic legs (Fig. 1.9; A, E)……2
- Antennae much longer than prothoracic legs (Fig. 1.9, C)……*Antispila* Hübner, 1825
2. Mandibles do not reach caudal edge of labrum (Fig. 1.9, A). Prothoracic femora not wider than prothoracic legs (Fig. 1.9, A)……*Antispilina* Hering, 1941
- Mandibles reach caudal edge of labrum and are pointed (Fig. 1.9, F). Prothoracic femora evidently wider than prothoracic legs (Fig. 1.9, E)...……*Heliozela* Herrich-Schäffer, 1853

Key to species:

1. Head with long upward-pointing setae. Labrum tapered. Maxillary palpi separated from maxillae (Fig. 1.9, G)……*H. resplendella* (Stainton, 1851)
- Head lacks long upward-pointing setae. Labrum rounded. Maxillary palpi adjacent to maxillae (Fig. 1.9; E, H)……2
2. Labium more than three times longer than labrum. Proboscis considerably overlaps prothoracic femora. Frons more obsolutely rounded (Fig. 1.9, H)……*H. hammoniella* Sorhagen, 1885
- Labium barely two times longer than labrum. Proboscis slightly overlaps prothoracic femora. Frons more pointed-rounded (Fig. 1.9, E)……*H. sericiella* (Haworth, 1828)

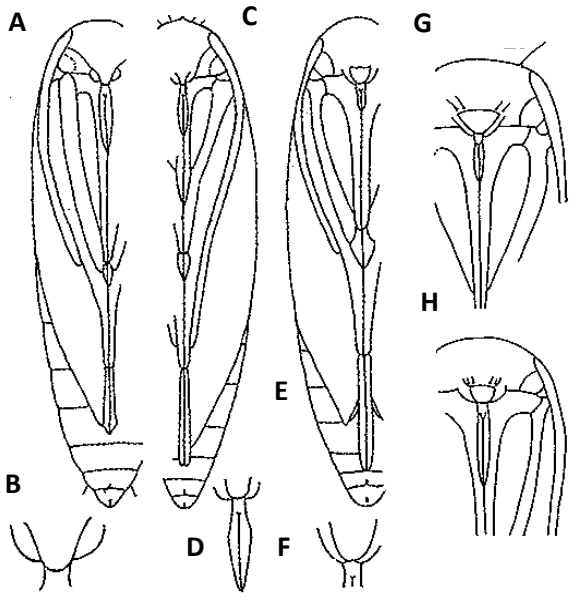

Figure 1.9: Pupae of Heliozelidae, *Antispilina ludwigi* (A, B); *Antispila treitschkiella* (C, D); *Heliozela sericiella* (E, F); *H. resplendella* (G) and H. *hammoniella* (H). Ventral view (A, C, E, G, H) and, labrum and vicinity (D, F). Adapted from Patočka & Turčáni (2005).

1.4. Biology, habits, geographical distribution and host plants

In the course of time, phytophagous insect larvae, such as those of heliozelids, have evolved peculiar specialized feeding habits which are, in most cases, characteristic of the species concerned (Hering 1951). Early Lepidoptera evolved an endophytic life style once they left the moist soil whilst exposed feeding behaviours only evolved later (Forbes 1923; Nielsen & Common 1991; Menken *et al*. 2009; Regier *et al*. 2015).

By means of an extensible piercing ovipositor females insert eggs singularly into plant tissue (Stehr 1992; Davis 1998; Bernardo *et al*. 2015). After hatching, larvae excavate mines by the consumption of mesophyll (Fig. 1.10) (Bernardo *et al*. 2015). Stehr (1992) describes the apodal larvae as "serpentine miners" that create small blotch mines which are essentially feeding channels within the parenchymal tissues of plants (Fig. 1.11) (Hering 1951). The cavity formed within the leaf serves as both living and feeding quarters for insect larvae (Hering 1951). Furthermore, larvae utilize the air within plant tissues for respiration (Nielsen & Common 1991). By adopting this feeding mechanism, they are able to isolate themselves from the outside, providing a relatively safe environment. Concealed feeding environments provide protection from desiccation, rapid temperature fluctuations and other physical

hazards. It does not, however, protect larvae against host specific parasitoids and predators, such as birds (Nielsen & Common 1991).

The number of larval instars is normally four or five but may vary between species (Hering 1951). The cremaster of a pupa frequently provides a means of species differentiation, especially amongst those that appear similar and cannot be distinguished from their mines by themselves (Hering 1951). Final instars form protective oval casings from the epidermal layers of the leaf before pupation (Scoble 1992).

The larvae of most heliozelids produce voluminous frass (Forbes 1923) which often provides a unique feeding pattern within the leaf. As a result, the mine produced by any leafmining insect can, in most cases, be used to characterize the order, family and even the genus of the particular leafminer (Fig. 1.12) (Hering 1951; Vári 1961). Due to the fact that the feeding patterns of leaf mining insects are particularly unique, it is, in almost all cases, easier to identify the genus and the species of the insect responsible from its unique feeding pattern (the study of which is referred to as hyponomology) than from the minute and subtle characteristics of the larvae and adults of the species concerned. In addition, this means of identification is often preferred, as it reduces the need to identify adults on the wing which may be troublesome when generations per year are few (Hering 1951) or when adult moths fail to emerge from pupae.

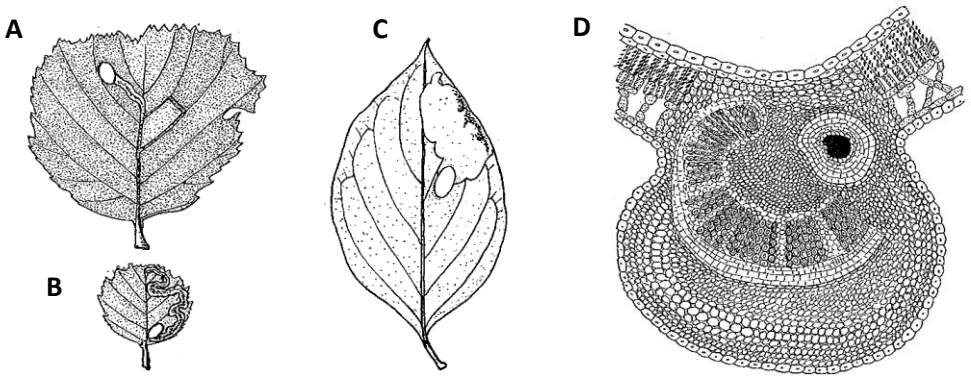

Figure 1.10: Heliozelid leafmines (A-C) and a cross-section thereof (D). Abandoned feeding channels, normal channel (A) and abnormal channel (B) created by *Heliozela resplendella* in *Alnus* sp.; blotch mine of *Antispila metallella* Hb. (C) in *Cornus* sp.. A cross-section of the leaf mid-rib (*Alnus* sp.) through a mine channel (filled with callus) by *Heliozela resplendella* Stt. (first generation) (D). Adapted from Hering (1951).

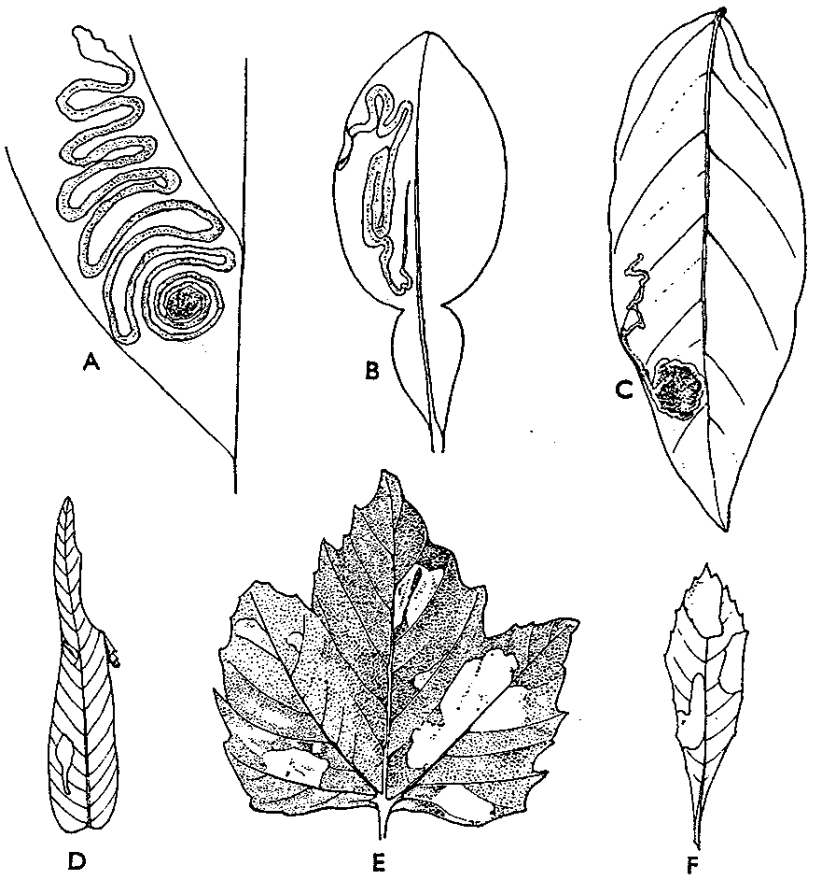

Figure 1.11: Larval leafmines of the Lepidoptera: A, Stigmellidae, *Stigmella arifoliella* Klim on *Rumex arifoliellus* Allioni; B, Lithocolletidae, *Phyllocnistis citrella* Stt. on *Citrus* sp.; C, *Acrocercops dessicata* Meyr. on *Ficus glomerata*; D, *Caloptilia phasianipennella* Hb. on *Polygonum* sp.; E, *Lithocolletis plantani* Stgr. on *Platanus* sp.; F, Lyonetiidae, *Lyonetia ledi* Wck. on *Myrica gale* L.. Taken from Bourgogne (1951).

Similar to other leafmining species, all instars are considered to be miners with the exception of the final larval instar (Stehr 1992). The final instar experiences a change of instinct (Hering 1951) and constructs an oval case from the epidermal layers of a mined leaf (Scoble 1992). Flat, lenticular casings are constructed by cutting sections from the upper and lower epidermal layers of the mine which are consequently lined and bound with silk to form a firm covering or a type of cocoon (Holloway *et al*. 1987; Stehr 1992). Most larvae have the ability to suspend themselves on a silken strand, leaving the infested leaves before pupation to attach themselves to the bark or leaves of the host plant, surrounding vegetation or other support (Bernardo *et al*. 2015). Abandoned leafmines are characterized by the presence of an oval cavity where the larvae excised the pupal casings (Bernardo *et al*. 2015). The larvae overwinter inside the cocoon throughout colder winter months and it is believed that the larvae only pupate a few weeks prior to emergence (Stehr 1992).

The geographical distribution of endophagous or leafmining insects is dependent on the distribution of their host plants (Hering 1951). It is often the case, however, that the distribution of the insect is less extensive than that of their host plants. Hering (1951) quotes the example of *Antispilina ludwigi* Hering, which feeds on the widespread *Polygonum bistorta* L., native to Europe and North and West Asia. The moth itself, however, is confined to Poland, Germany and the Czech Republic. Interestingly, the European and North American *Antispila* species feed on Cornaceae and Vitaceae which are, phylogenetically, widely separate. However, common to both these families is a pigment described as "wine-red" (anthocyanin), a pigment distinct from all other related red pigments referred to as "beet-red" (betalain) (Hering 1951). A variety of other host plants for the Heliozelidae have been identified (Table 1.1).

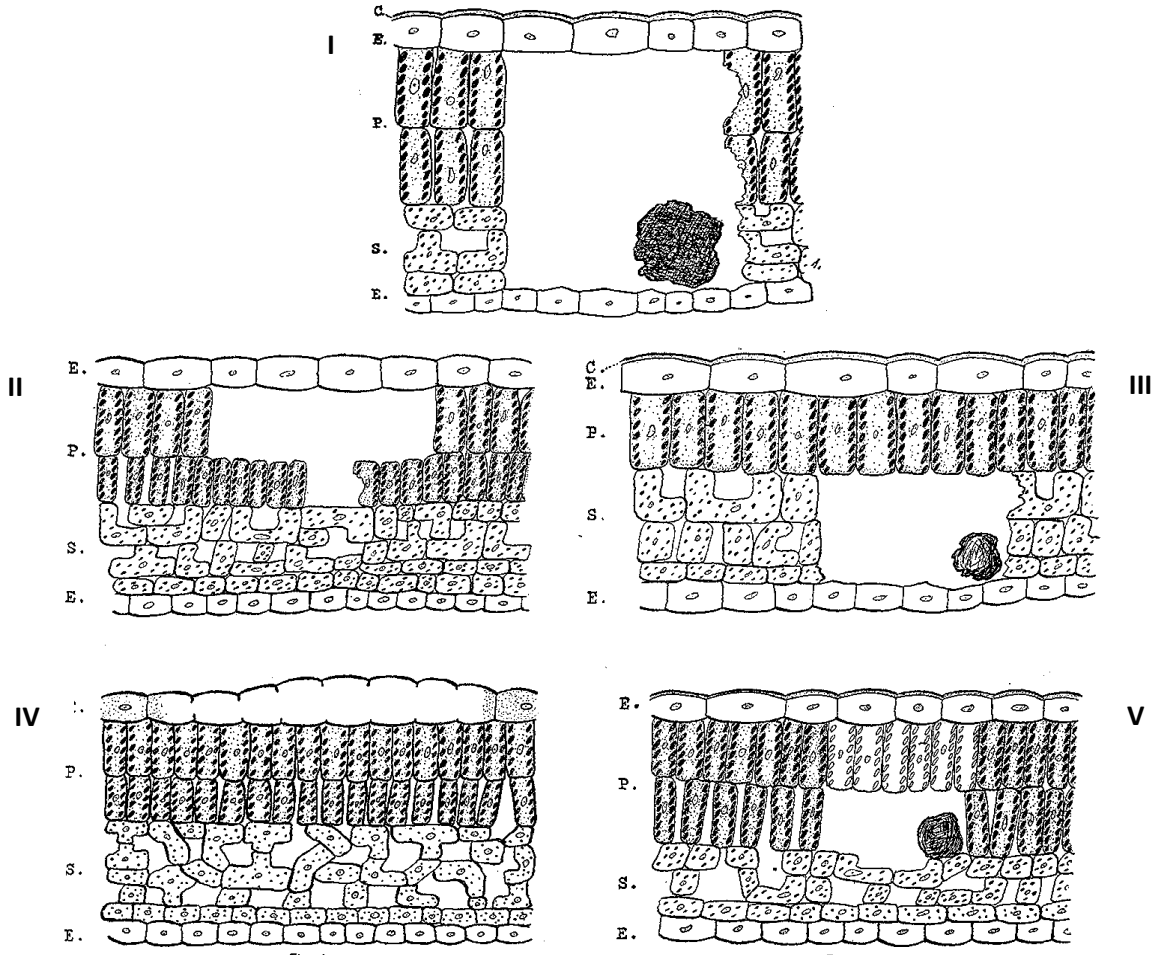

Figure 1.12: Cross-section of mines through leaf tissue. A full-depth mine (I), an upper surface mine (II), a lower surface mine (III) an upper epidermal mine of *Phyllocnistis* (IV) and an inter-parenchymal mine by *Phytomyza affinis* Fall. on Cirsium (V). C = cuticle, E = epidermis, P = palisade parenchyma, S = spongy parenchyma. Frass remnants are represented in I, III and V. Adapted from Hering (1951).

Table 1.1: Summary of recorded host plants affected by Heliozelidae based on findings in available literature sources.

PLANT FAMILY	PLANT SPECIES	REGION/LOCALITY	HELIOZELID SPECIES	SOURCE
Anacardiaceae	*Lannea coromandelica*	Kawar, India	*Holocacista micrarcha* (Meyrick)	Nieukerken & Geertsema (2015)
		Pusa, India	*Holocacista pariodelta* (Meyrick)	
	Lannea discolor	South Africa	Unknown species	
Balsaminaceae	*Impatiens clavigera*	Vietnam	Unknown species	
Betulaceae	*Alnus glutinosa*	Britain	*Heliozela resplendella* (Stainton)	Patočka & Turčáni (2005); Hering (1951); Browne (1968)
	Betula sp.	North America	*Antispila argentifera* Braun	Eiseman & Charney (2010)
		Unknown	*Heliozela hammoniella* Sorhagen	Patočka & Turčáni (2005)
Combretaceae	*Terminalia pruniodes*	South Africa	Unknown species	Nieukerken & Geertsema (2015)
Cornaceae	*Cornus brachypoda*	Japan	*Antispila corniella* Kuroko	Kuroko (1961)
		Japan	*Antispila hikosana* Kuroko	
		Japan	*Antispila purplella* Kuroko	
	Cornus canadensis	North America	*Antispila freemani* Lafontaine	Eiseman & Charney (2010); Ferguson (1975)
	Cornus controversa	Japan	*Antispila corniella* Kuroko	Kuroko (1961)
		Japan	*Antispila hikosana* Kuroko	
		Japan	*Antispila purplella* Kuroko	
	Cornus mas	Central Europe	*Antispila treitschkiella* (Fischer von Röslerstamm)	Patočka & Turčáni (2005)
	Cornus sp.	North America	*Antispila aurirubra* Braun	Braun (1915)
		Unknown	*Antispila metallella* Hübner	Hering (1951)
	Nyssa sylvatica	North America	*Antispila nysaefoliella* Clemens	Eiseman & Charney (2010)
Dilleniaceae	*Hibbertia* sp.	West Australia, Australia	Unknown species	Nieukerken & Geertsema (2015)

Ericaceae	*Arbutus* sp.	North America	*Coptodisca arbutiella* Busck	Powell & Opler (2009); Furniss & Carolin (1977)
	Kalmia angustifolia	North America	*Coptodisca kalmiella* Dietz	Weiss & Beckwith (1921)
Fagaceae	*Quercus petraea*	Britain	*Heliozela sericiella* (Haworth)	Patočka & Turčáni (2005); Browne (1968)
	Quercus robur	Britain	*Heliozela sericiella* (Haworth)	Browne (1968)
	Quercus serrata	Japan	*Heliozela limbata* Lee, Hirowatari & Kuroko	Lee *et al*. (2006a)
Geraniaceae	*Pelargonium citronellum*	Western Cape, South Africa	*Holocacista varii* (Mey)	Nieukerken & Geertsema (2015)
	Pelargonium cucullatum	Western Cape, South Africa	*Holocacista varii* (Mey)	
	Pelargonium hispidum	Western Cape, South Africa	*Holocacista varii* (Mey)	
	Pelargonium panduriforme	Western Cape, South Africa	*Holocacista varii* (Mey)	
Hamamelidaceae	*Distylium racemosum*	Japan	*Antispila distyliella* Lee, Hirowatari & Kuroko	Lee *et al*. (2006b)
Hydrangeaceae	*Hydrangea arborescens*	North America	*Antispila hydrangaeella* Chambers	Nieukerken *et al*. (2012); Eiseman & Charney (2010)
	Hydrangea macrophylla	Japan	*Antispila hydrangifoliella* Kuroko	Kuroko (1961)
	Hydrangea petiolaris	Japan	*Antispila hydrangifoliella* Kuroko	
Juglandaceae	*Carya illinoinensis*	North America	*Coptodisca lucifluella* (Clemens)	Western Pecan Growers Association (2011)
Myrtaceae	*Eucalyptus* sp.	Australia	*Helizela prodela* Meyrick	Browne (1968)
	Eugenia sp.	North America	*Antispila eugeniella* Busck	Eiseman & Charney (2010); Busck (1900)
	Syzygium cumini	India	*Antispila anna* Meyrick	Browne (1968)

Plumbaginaceae	*Dyerophytum indicum*	Fujairah, United Arab Emirates	Unknown species	Nieukerken & Geertsema (2015)
Polygonaceae	*Bistorta officinalis*	Central Europe	*Antispilina ludwigi* (Hering)	Patočka & Turčáni (2005)
Roseaceae	*Malus* sp.	North America	*Coptodisca splendoriferella* (Clemens)	Holloway *et al*. (1987)
Rubiaceae	*Lasianthus* Jack sp.	Kalimantan Timur, Indonesia	*Holocacista* sp.	Nieukerken & Geertsema (2015)
		Sabah, Malaysia	Unknown species	
	Morinda jasminoides	Queensland, Australia	Unknown species	
	Paederia foetida	Taiwan	Unknown species	
	Psychotria dalzellii	Kawar, India	*Holocacista selastis* (Meyrick)	
	Psychotria simmondsiana	Queensland, Australia	Unknown species	
Theaceae	*Cleyera japonica*	Japan	*Antispila cleyerella* Lee, sp. nov.	Lee *et al*. (2006c)
Vitaceae	*Ampelopsis brevipedunculata*	Japan	*Antispila ampelopsia* Kuroko	Kuroko (1961)
		Japan	*Antispila orbiculella* Kuroko	
	Cayratia trifolia	India	*Antispila argostoma* Meyrick	Nieukerken *et al*. (2012)
	Cissus antarctica	Australia	Unknown species	
	Cissus cornifolia	South Africa	*Holocacista salutans* (Meyrick)	Nieukerken & Geertsema (2015)
	Cissus integrifolia	South Africa	Unknown species	
	Leea indica	Kalimantan Timur, Indonesia	Unknown species	
	Parthenocissus quinquefolia	North America	*Antispila ampelopsifoliella* Chambers	Nieukerken *et al*. (2012)
		North America	*Heliozela aesella* Chambers	
	Parthenocissus quinqueguttella	North America	*Antispila* cf *viticordifoliella* Clemens	
	Parthenocissus sp.	North America	*Antispila* cf *viticordifoliella* Clemens	
	Parthenocissus tricuspidata	Japan	*Antispila iviella* Kuroko	Kuroko (1961)
	Rhoicissus digitata	South Africa	*Holocacista salutans* (Meyrick)	Nieukerken & Geertsema (2015)
		South Africa	*Holocacista* sp.	
	Rhoicissus revoilii	South Africa	*Holocacista salutans* (Meyrick)	
	Rhoicissus tomentosa	South Africa	*Holocacista salutans* (Meyrick)	
		South Africa	Unknown species	
	Rhoicissus tridentata	South Africa	Unknown species	

Vitaceae	*Vitis aestivalis*	North America	*Antispila* cf *isabella* Clemens	Nieukerken *et al*. (2012)
		North America	*Antispila oinophylla* Nieukerken & Wagner	
	Vitis arizonica	North America	*Antispila voraginella* Braun	
	Vitis coignetiae	Japan	*Antispila inouei* Kuroko	
	Vitis coignetiae	Japan	*Antispila tateshinensis* Kuroko	Nieukerken *et al*. (2012)
		Japan	*Antispila uenoi* Kuroko	
	Vitis flexuosa	Japan	*Antispila ampelopsia* Kuroko	Kuroko (1961)
	Vitis labrusca	North America	*Antispila oinophylla* Nieukerken & Wagner	Nieukerken *et al*. (2012)
	Vitis labruscana	Japan	*Antispila inouei* Kuroko	
		Japan	*Antispila uenoi* Kuroko	
	Vitis riparia	North America	*Antispila* cf *isabella* Clemens	
		North America	*Antispila oinophylla* Nieukerken & Wagner	
		North America	Unknown species	
	Vitis sp.	India	*Antispila aristarcha* Meyrick	
		India	*Antispila isorrhythma* Meyrick	
		North America	*Heliozela aesella* Chambers	
	Vitis vinifera	Italy	*Antispila oinophylla* Nieukerken & Wagner	
		Malta	*Holocacista rivillei* (Stainton)	Nieukerken & Geertsema (2015)
		Western Cape, South Africa	*Holocacista capensis* Nieukerken & Geertsema	
	Vitis vulpina	North America	*Antispila oinophylla* Nieukerken & Wagner	Nieukerken *et al*. (2012)
		North America	*Antispila viticordifoliella* Clemens	
		North America	*Heliozela aesella* Chambers	

Several leafmining Lepidoptera are important agricultural pests, such as *Aproaerema modicella* Deventer (Gelechiidae) on groundnut, soybean and lucerne in Africa and South and East Asia (Shanower *et al*. 1993; du Plessis 2003); *Tuta absoluta* Meyrick (Gelechiidae) on tomatoes in West Asia, South America, North Africa and Europe (Siqueira *et al*. 2001; Siqueira *et al*. 2007; Zanuncio *et al*. 2005; Caparros Medigo *et al*. 2012; Zappala *et al*. 2012); *Phyllocnistis citrella* Stainton (Gracillariidae) on citrus in North America, North and South Africa, Asia and Australia (Villanueva-Jiménez & Hoy 1998; Villanueva-Jiménez *et al*. 2000); *Antispila nysaefoliella* Clemens (Heliozelidae) on tupelo (sour gum) in North America; *Coptodisca splendoriferella* Clemens (Heliozelidae) on apple in North America (Holloway *et al*. 1987); *Leucoptera coffeella* Guérin-Méneville (Lyonetiidae) and *L. caffeina* Washburn (Lyonetiidae) on coffee beans in South America and West Africa, respectively (Fragoso *et al*. 2002). *Holocacista rivillei* Stainton (Heliozelidae), a leafminer native to Europe, has caused minor damage to cultivated grapevines (*Vitis vinifera* L.) in the past (Nieukerken *et al*. 2012).

Three unexpected cases of leafminer infestations occurring on grapevines have been reported in the last three decades. Two leafminers native to North America, *Phyllocnistis vitegenella* Clemens (Gracillariidae) and *Antispila oinophylla* Nieukerken & Wagner (Heliozelidae) have more recently invaded Northern Italy and other parts of Europe (Nieukerken *et al*. 2012). In another case, a native leafminer described as *Antispila uenoi* Kuroko has been reported on cultivated grapevines in Japan (Nieukerken *et al*. 2012).

The African Heliozelidae fauna was restricted to three known species described from South Africa. The species include *Antispila argyrozona* Meyrick, 1918, *A. salutans* Meyrick, 1921 and *Antispilina varii* Mey, 2011. Only the latter originates from the leafmines of a known host, *Pelargonium* L.'Hérit (Western Cape). Nieukerken & Geertsema (2015) diagnose and redescribe the *Holocacista* genus in a recent study whilst providing notes on other South African Heliozelidae.

1.5. *Holocacista capensis*

The latest compendium of Insects of Cultivated Plants and Natural Pastures (Prinsloo & Uys 2015) in Southern Africa lists 35 grapevine pests. Of those, Cossidae, Tortricidae, Sphingidae, Agaristidae and Noctuidae have been identified as prominent pest families within Lepidoptera (Nel 1983). Absent from this list is a native heliozelid moth.

In January, 2012, high abundances of an unknown grapevine leafminer were observed in a table grape vineyard by Dr J. de Waal (DOW AgroSciences), in the Windmeul area, close to Paarl in the Western Cape, South Africa. Collected samples were analysed by Prof. H. Geertsema, who tentatively identified the damage as those of *Antispila* Hübner, 1825, based on similarities in infestation behaviour and morphology. Subsequent field visits indicated that infestation was severe in table grape vineyards in Paarl and its surroundings. In many cases, cocoons were found rooted to grape bunches (the pupae, therefore, form a surface contaminant on grape berries), but also to foliage, stems and trellises. As a result, the leafminer is of primary quarantine importance for the export table grape industry. Additional larval samples were collected from infested blocks and sent to Dr van Nieukerken (Naturalis Biodiversity Centre, Leiden, the Netherlands) to confirm identification.

Further investigation showed that the very first record of the newly discovered leafminer (described then as *Antispila* species), outside of the Western Cape, dated back to 1950 in Pretoria where Dr Lajos Vári bred the miner from an ornamental vine growing in his garden (Nieukerken & Geertsema 2015). Several other cases were reported from Vaalhartz Research Station (Northern Cape) and Roodeplaat (Pretoria) in 1980 and 1990, respectively. Miss S. Marais collected samples, found for the first time in the Western Cape, in March 1998, from ornamental vines in Oudtshoorn. A closer investigation into the undescribed heliozelids collected by Dr Vári indicated that South Africa hosts a small, but diverse fauna of heliozelids (Nieukerken & Geertsema 2015).

Since its discovery in 2012, several other cases of the leafminer were reported on commercial and ornamental grapevines in the Western Cape. These areas include Woodstock (Cape Town), Stellenbosch, Somerset West, Simondium, Wellington and Robertson. Relatively

high infestations were even reported from the Brits region in Gauteng (J. de Waal *pers. comm.*; H. Geertsema *pers. comm.*).

Pilot trials and general observations made throughout the remainder of the 2010/2011 and 2011/2012 growing seasons indicate that the peak of the moth season occurred near the end of the grapevine growing season and, in most cases, after harvest. Several studies have explored the negative effects of leafmining activities on the photosynthetic ability of host plants (see Johnson *et al*. 1983; Parrella *et al*. 1985; Schaffer *et al*. 1997; Wagner *et al*. 2008). Unlike many other leafmining Lepidoptera (Hileman & Lieto 1981), the effect of the mines, caused by the feeding habits of the new leafminer, on the photosynthetic ability of a grapevine is not yet known, although it appears to be limited (Nieukerken & Geertsema 2015). These assumptions suggest that the leaf damage does not affect the quality or quantity of the fruit due to the fact that leafminer populations are relatively low and damaged leaves are continuously replaced by fresh foliage throughout the growing season. Despite the fact that there appears to be no negative effect on the physiology of the plant, premature abscission could possibly be experienced by severely infested grapevines (Gilbert *et al*. 2004). No single preference for a particular cultivar has been identified, although field observations have suggested that red cultivars are more susceptible to leafminer infestation (Nieukerken & Geertsema 2015).

Nieukerken & Geertsema (2015) recently described the new leafminer, collected from vineyards in the Western Cape, as *Holocacista capensis* and revised its identity and taxonomy. In addition, several of South Africa's known heliozelids were recombined with *Holocacista*.

The morphology of *H. capensis* has been described as follows (Nieukerken & Geertsema 2015):

Adults

Very small moths, wingspan ca. 3.9 – 4.9 mm. Wings characterised by a pattern of metallic-silvery spots. Appressed, metallic, silvery-white scales cover the head and face. Scales are more brownish-grey on the vertex. Palpi porrect. White scales cover the base of the

proboscis. Antennae with 16 segments, ringed, each flagellomere with a basal fuscous scale ring and apical white scales on the upper side, all scales on underside are white. A triangular dorsal spot on the medial forewing extends towards the centre of the wing. Triangular spot beyond centre is always separate. The legs are grey whilst tarsi are mostly yellowish-white in colour, especially on the ventral side. Ground colouration of thorax and forewings is grey-brown, slightly irrorate. Hindwings are pale grey. The underside of wing is fuscous, white spots are visible. The fringe line on forewing is distinct and is demarcated by dark-tipped scales.

Males

The silver-white pattern on the forewing consists of a triangular dorsal spot located one-fourth from the forewing base and is, in most cases, associated with a minor spot at the costa that may be absent or joined to the dorsal spot. The abdomen and vestiture on the external genitalia is lead-coloured.

Females

Scales on the forewing are almost uniformly dark fuscous with a purplish tinge (darker, velvety wing colour) creating clear contrast between the dark scales and the silvery-white wing pattern. The first costal and dorsal spots are joined to form a narrow fascia (wider at dorsum). Scales on the fringe of the wing form cilia with slightly paler bases. The abdomen is almost black and narrowly pointed posteriorly.

Larvae (including most other heliozelids)

Larvae are usually characterised by a dark, prognathous head capsule and a yellow or whitish body. Prolegs are absent, however, ambulatory and fused ventro-medial calli are present on abdominal segments T2-3 (ventral and dorsal) and A3-6, respectively. Feeding larvae develop through four feeding instars which is followed by a fifth, non-feeding, larval instar. It is this final instar that constructs the case in which it pupates.

It is considered that *H. capensis* is in fact a native leafminer of various indigenous Vitaceae such as *Rhoicissus tomentosa*, *R. digitata*, *R. tridentata* and *Cissus cornifolia*. Thus far, studies by Nieukerken & Geertsema (2015) have only been able to report the presence of *H. capensis* in a single population of *R. digitata* from the Wilderness district in the Western

Cape. It is hypothesized further that the host shift from native Vitaceae to *Vitis vinifera* L. could have taken place in the Western Cape specifically. The presence of the leafminer in Roodeplaat and Vaalhartz Research Station is most likely the result of transport of root stock and scions from large-scale nurseries in the Western Cape, more specifically the Wellington and Paarl regions, to the more northern parts of the country.

Damage symptoms by this leafminer are completely unique as they include the presence of small to medium-sized foliar blotch mines (mature mines usually ca. 15 mm in length) and the presence of their small cocoons attached to grapevine berry bunches, vine foliage, stems and to trellises. Any form of the insect present on export fruit can potentially lead to the rejection of fruit intended for export as the insect is regarded as a phytosanitary risk due to the fact that cocoons attached to grape bunches are undesirable to the importing country.

In the past, growers, labourers and pest control agents have suggested that incidents of cocoons attached to berries intended for export, observed before 2012, were mistaken for discarded scale insect exuviae or plant debris. In the past two grapevine growing seasons, growers found that the removal of cocoons attached to harvested berries (on bunches intended for export) by removal of affected berries or brushing the cocoons from berries, was often tedious and labour intensive. In the field, a large abundance of descending cocoons (pupae suspended from silken threads) are observed in heavily infested blocks throughout the grapevine growing season. During the 2012 harvest, infestation was so immense on a farm in Paarl, that the suspended cocoons (potential pupae suspended from silken threads) of these insects had to be cleared from the radiators of the tractors before moving on to new rows (H. Geertsema *pers. comm*.).

1.6. *Vitis vinifera*

The grape, *Vitis vinifera* (Vitaceae), is thought to have originated in areas surrounding the Caucasus and has been grown for thousands of years by mankind (Baker & Waite 2003). The introduction of *V. vinifera* to Mesopotamia occurred as far back as 6000 BC, making grapes one of the earliest cultivated fruits on earth. Later, grapes were introduced to Syria, Greece and Egypt which resulted in the spread of grapes across Europe and the British Isles

after the conquest of the Middle East and Western Europe by the Romans (Baker & Waite 2003). The introduction of table grapes to South Africa was thought to have occurred at the Cape of Good Hope, in the late 1650's, to supply fruit to passing ships (Hurndall 2005).

Fresh table grapes and dried grapes account for approximately 34% of the 79 803 hectares of land planted to deciduous fruit trees in South Africa, followed by apples (29%) and pears (15%) (Key Deciduous Fruit Statistics, 2014). The remaining 22% is dedicated to the growth of peaches, plums, prunes, apricots and nectarines. Table grapes and dried grapes contribute approximately 7% of the R9.8 Bn turnover produced by deciduous fruit growers, second to pome fruit (73%) and stone fruit (20%) (Key Deciduous Fruit Statistics, 2014).

Within southern Africa there are five primary table grape producing regions, namely the Northern Provinces, the Orange River, the Olifants River, the Berg River and the Hex River Valley (Fig. 1.13). The Western Cape houses three of these table grape producing regions (Olifants River, Berg River and Hex River Valley) and, therefore, collectively produces the highest yield of table grapes than any other province (Table 1.2). In the 2013/2014 and 2014/2015 grapevine growing seasons, the Western Cape produced little over 62% of the national table grape intake (SATI Statistics Booklet 2014).

1.7. Pest management

Various chemical products have been used for the control of economically important leafmining pests. Studies done by Trumble (1985) and Weintraub & Horowitz (1998) found that avermectin, cyromazine and abamectin effectively reduced dipterous leafminer abundance on *Apium graveolens* L. (celery) and spinosyns (derived from fermented bacteria) have been known to control leafmining Lepidoptera (Zhao *et al*. 2002). Many publications have explored the efficacy of such chemicals in field and laboratory conditions on a variety of leafminers (e.g. Beattie *et al*. 1995; Rae *et al*. 1996a; Rae *et al*. 1996b; Villanueva-Jiménez *et al*. 2000; Zhao *et al*. 2002; Zhao *et al*. 2006; Lietti *et al*. 2005; Elbert *et al*. 2008). At present, no chemical pesticides are registered for the control of *H. capensis*.

In most commercial orchards, worldwide, insecticides are the main defence against leafminers and other insects (Maier 2001). Several publications have emphasised the need for appropriate Integrated Pest Management (IPM) programmes to reduce the reliance on chemical management which, in some cases, has led to pesticide resistance and the suppression of natural enemies (see Shanower *et al*. 1993; Maier 2001; Zhao *et al*. 2002; Zhao *et al*. 2006; Lietti *et al*. 2005).

Biological control of leafminers has been investigated as an alternative to chemical control (Maier 2001). Several parasitoids of *H. capensis* attack both larval and pupal life stages (H. Geertsema, *pers. comm*.). The presence of such parasitoids supports the notion that the moths have been present in the region for a considerable amount of time. Over the last few decades, the parasitoid wasps of *Phyllocnistis citrella* Stainton (Gracillariidae) have been well documented, several of which have been found to exert substantial control on the citrus leafminer (Smith & Hoy 1995; Peña *et al*. 1996; Pomerinke & Stansly 1998; Garcia-Marí *et al*. 2004; Elekçioğlu & Uygun, 2006). It has been suggested that incidents of leafminer damage symptoms, observed before 2012, were mistaken for discarded scale insect exuviae or plant debris according to recollections made by growers, labourers and pest control agents.

Mating disruption for populations of *Tuta absoluta* Povolny (Gelechiidae) and *Phyllocnistis citrella* Stainton (Gracillariidae) has been studied in greenhouse conditions, although further research is required to determine the efficacy of mating disruption for leafminers in outdoor field conditions (Mafi *et al*. 2005; Vacas *et al*. 2011). Entomopathogenic nematodes have also proven to be of value in terms of potential IPM strategies (e.g. Head *et al*. 2000; Batalla-Carrera *et al*. 2010).

The first identification of sex pheromone in *H. capensis* was recently reported by Wang *et al*. (2015). Using gas chromatography-mass spectrometry coupled with field experiments, (*Z*)-5-tetradecenal and (*Z*)-7-tetradecenal were identified as viable male attractants (Wang *et al*. 2015).

1.8. Aims and objectives

The aim of the present study was to conduct baseline studies to better understand the bio-ecology and distribution of *H. capensis* throughout the Western Cape, as well as to establish an appropriate monitoring system.

Objectives:

i) To determine the distribution of *H. capensis* throughout the Western Cape with special reference to the dominant table grape producing regions by the use of baited pheromone traps;

ii) to determine the seasonal cycle of adults using baited pheromone traps and the seasonal occurrence of larvae by the assessment of infested leaves;

iii) to develop an appropriate monitoring system for *H. capensis*, using an adaptation of the generic pest monitoring system formulated by de Villiers & Pringle (2007), and

iv) to conduct and test preliminary post-harvest controls using standard USDA approved post-harvest control protocols adopted for the control of False Codling Moth and Mediterranean Fruit Fly on table grapes.

The chapters that follow are structured as individual publications. Some repetition is, therefore, unavoidable.

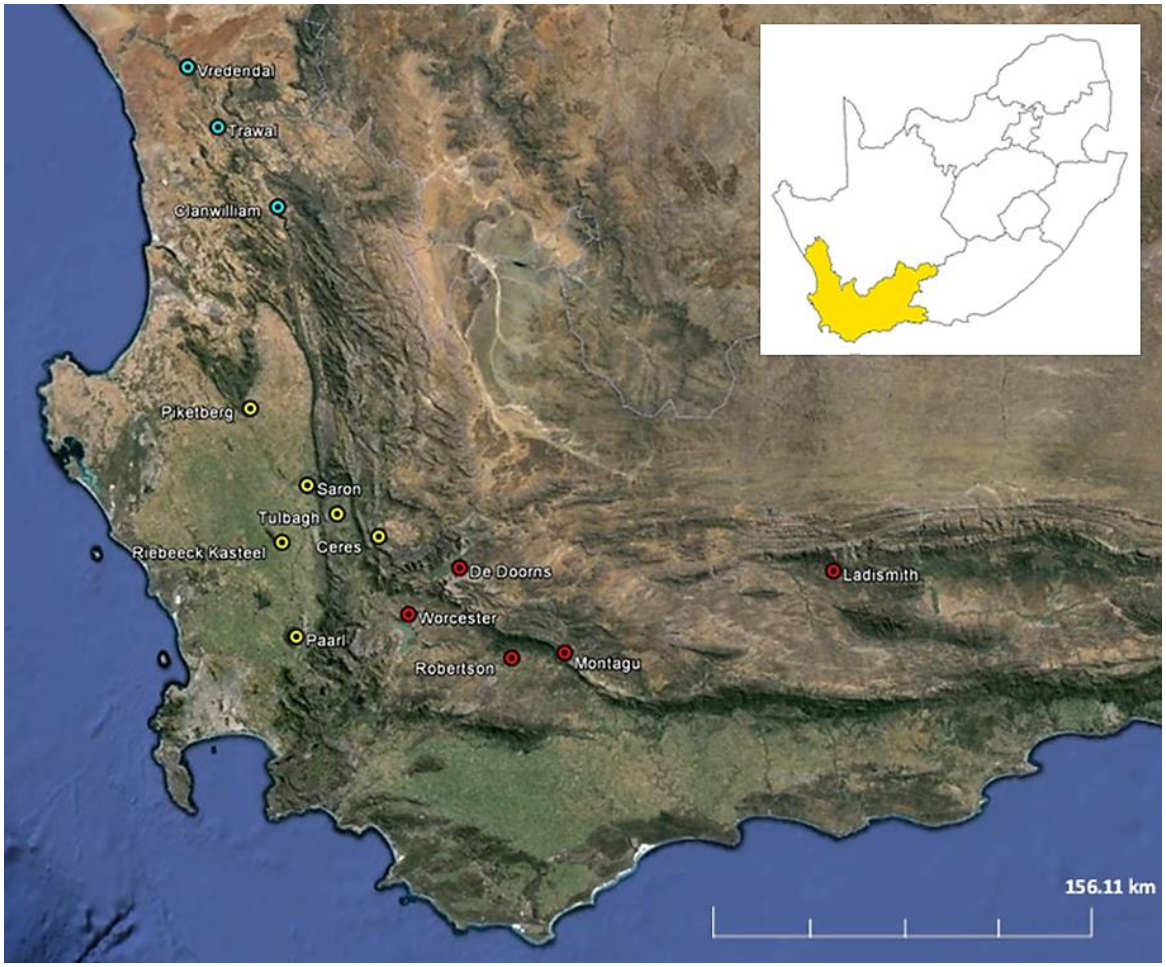

Figure 1.13: The primary table grape producing regions in the Western Cape, South Africa, the Olifants River (blue markers); the Berg River (yellow markers) and the Hex River Valley (red markers).

Table 1.2: Regional table grape intake (4.5 kg equivalent cartons) over five seasons in each of the table grape producing regions of southern Africa. Table adapted from SATI Statistics Booklet (2014).

REGION	**INTAKES PER SEASON**					
	2009/10	**2010/11**	**2011/12**	**2012/13**	**2013/2014**	**2014/15**
Northern Provinces	4 390 078	3 577 697	4 254 175	4 155 648	4 083 599	4 510 726
Orange River	16 765 935	13 942 172	16 825 188	16 039 382	15 118 961	17 686 725
Olifants River	1 887 890	1 778 367	2 271 646	2 725 942	3 121 056	3 788 287
Berg River	11 572 671	11 922 891	12 874 338	12 672 168	11 379 002	13 062 449
Hex River Valley	18 637 409	18 224 130	18 432 048	18 286 725	16 846 196	20 331 091

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Chapter 2

The bio-ecology of the Cape grapevine leafminer (*Holocacista capensis*) in the Western Cape, South Africa

2.1. Introduction

A native leaf-mining heliozelid, recently described as *Holocacista capensis* Nieukerken & Geertsema (2015), has since 2012 raised concerns by the table grape industry in the Western Cape, South Africa. The study by Nieukerken & Geertsema (2015) concluded that this newly discovered moth is closely related to *H. salutans* (Meyrick, 1921) comb. n. (ex *Antispila*), another native leafminer known to feed on a variety of native *Rhoicissus* and *Cissus* species. The very first record of *H. capensis* (described then as an *Antispila* species), outside of the Western Cape, dates back to 1950 in Pretoria where the leafminer was bred from an ornamental vine by Dr L. Vári. Several other cases of the moth's presence have been reported since 1950 from the Vaalhartz Research Station (Northern Cape) and Roodeplaat (Pretoria) in 1980 and 1990, respectively. The leafminer was recorded for the first time in the Western Cape in March 1998 from ornamental vines in Oudtshoorn.

From the reports and samples collected since 1950 it was clear that the distribution of *H. capensis* was relatively widespread throughout South Africa (Nieukerken & Geertsema 2015) (Fig. 2.1). Since the first official report of the leafminer on vine grapes in 2012, several reports of its presence on *Vitis vinifera* L. (Vitaceae) have been reported from Cape Town, Stellenbosch, Simondium, Robertson, Wellington in the Western Cape and the Brits region in Gauteng. In addition, Nieukerken & Geertsema (2015) reported an association between *H. capensis* and a local population of native *Rhoicissus digitata* in the Wilderness region. The main presence of the Cape grapevine leafminer is, therefore, confined to the Western Cape. Field visits made throughout the Western Cape have, to date, indicated severe cases of infestation by *H. capensis* in table grape vineyards in Paarl and Wellington.

Figure 2.1: The known distribution of *Holocacista capensis* (black markers) in South Africa prior to 2014 (Nieukerken & Geertsema 2015).

Three separate cases of leafminer infestations found for the first time on cultivated grapevines (*Vitis vinifera* L.) have been reported in other regions throughout the globe, over the last three decades. Of the infestations, two leafminers native to North America, *Phyllocnistis vitegenella* Clemens (Gracillariidae) and *Antispila oinophylla* Nieukerken & Wagner (Heliozelidae) have more recently invaded Northern Italy and other parts of Europe (Nieukerken *et al*. 2012). In another case, a native leafminer described as *Antispila uenoi* Kuroko was reported on cultivated grapevines in Japan (Nieukerken & Geertsema 2015).

This study aims to gain greater insights into the overall biology of *H. capensis* and its distribution throughout the Western Cape. This involved the investigation of a variety of biological aspects such as optimal rearing temperatures, associated parasitoids and predators as well as spatial distribution and preferences within the agricultural context. Emphasis will be placed on assessing the fluctuations in adult and larval populations throughout the grapevine growing seasons as well as understanding their overall distribution and spatial preferences in the agricultural and regional landscape. The overarching goal of this

investigation is to contribute to the current knowledge on the biology of the pest, establish potential identification techniques and assist in the development of appropriate management strategies associated with biologically based IPM strategies. The spatial and temporal distritubiton of the adult and immature stages of the moth were thus monitored over two grapevine growing seasons (2013/2014 – 2014/2015), while various abiotic factors were assessed in relation to moth activity patterns. For the purposes of this thesis, all leafminers found in the vineyards were assumed to be *H. capensis*, as identifying species pest complexes were out of the scope of this project.

2.2. Materials and methods

2.2.1. Study sites

This study was conducted in the Drakenstein region of the Cape Winelands on three farms in the districts of Paarl and Wellington in the Western Cape Province of South Africa [namely Optenhorst (33°43'03.8"S 18°57'55.8"E), Monte Cristo (33°40'21.1"S 18°54'05.3"E) and St Malo (33°35'52.7"S 18°58'46.0"E)]. Following the sampling methods of de Villiers & Pringle (2007), two to three blocks of vine cultivars were selected at each farm. Within each block, 20 evenly distributed plots (internal plots), each consisting of five vines, were chosen along five equally distributed rows. An additional plot was established at the end of each row sampled (external plots). Each block was sampled fortnightly.

Ad hoc sampling was similarly conducted in Malmesbury (Nieuwedrift Vineyards, 32°58'45.1"S 18°44'51.7"E), Porterville (Houtconstant, 33°00'30.4"S 19°00'54.0"E), Tulbagh (Olyfboom, 33°17'28.4"S 19°05'25.1"E), Riebeeck Kasteel (Het Vlock Casteel, 33°23'22.2"S 18°53'36.1"E) and Paarl (de Heuvel, 33°40'00.2"S 18°55'14.0"E).

2.2.2. Ecology

Field observations of laboratory and field populations were used to obtain a basic outline of the life cycle of *Holocacista capensis*. To sustain laboratory populations kept under relatively stable conditions (room temperature), cocoons from all study site localities were collected at random and placed in a rearing chamber (a large perspex box). *Rhoicissus*

digitata was used to sustain the population during the colder winter months, whilst an ornamental variety of *Vitis vinifera* was used in conjunction with *R. digitata* in the warmer summer months. Plant growth and survival was aided by the use of a standard fluorescent grow lamp (Sylvania grow-lux, F15W) coupled to an electronic ballast (Daro, T8-20W) and mounted above the perspex roof. In the winter of 2014, an additional perspex box was placed outside to observe overwintering behaviour/strategies. Various behavioural and structural aspects were recorded by the use of a Leica stereo microscope (e.g. abandoned mines, emerging parasitoid wasps and feeding larvae).

Sexing of cocoons was explored as a means of a potential sexing mechanism. To verify the sexes of the cocoons, differences between adult male and female moths had to be established on a macroscopic level. Sexed cocoons were monitored individually (aerated microfuge tubes were used to house cocoons separately) and the sex of an individual was verified after eclosion. Approximately 328 cocoons (collected from the field) were sexed prior to eclosion based on the size and shape of the cocoons. All parasitoids, that emerged from all cocoons and larval mines collected throughout the study, were collected, stored in alcohol and identified to family level.

A pilot trial was conducted on 100 cocoons to establish an optimal rearing temperature. Twenty fresh cocoons were exposed to five different rearing temperatures (15°C, 20°C, 25°C, 30°C and 35°C) in temperature controlled incubators (MRC LE-509, Holon, Israel) and mortality at each temperature setting was recorded.

To better understand overwintering strategies, bark (ca. 400 g) and leaf litter (ca. 200 g) samples were collected randomly from all blocks within St Malo on the 11th of June 2014. Bark and leaf samples were stored in aerated containers and placed outside during the winter. In the following season, samples were sorted and adult moth abundance recorded.

2.2.3. Seasonal monitoring

2.2.3.1. Monitoring the seasonal occurrence of larvae

One leaf per vine (those mentioned in section 2.2.1.) was examined for damage symptoms associated with *Holocacista capensis*. In the case of the additional external plots, five leaves were collected at random. Collected leaves were placed in plastic sleeves and taken to the laboratory where they were stored (prior to analysis) in a cool room. With the aid of a microscope and a reading lamp, the number of cocoons, excavation sites (holes, also referred to as matured mines) and larvae per leaf were recorded (Appendix 1, Table 1.a). In the 2014/2015 grapevine growing season, several other variables were included. Based on the Total Surface Area (S), leaves were classified into three different size classes: small ($S \leq 60$ mm^2), medium ($60\ mm^2 < S < 150\ mm^2$) and large ($S \geq 150\ mm^2$), based on the classification system used by H. Geertsema (*pers. comm.*) in his preliminary studies (2012 – 2013) conducted in the Paarl region. Trellis posts situated on each end and in the middle of each row (i.e. three trellis posts) were inspected for cocoons.

Monitoring was carried out over a period of 15 months, from February, 2014 to May, 2015. During the two grapevine growing seasons, leaves were sampled (to monitor fluctuations in larval populations) until leaf senescence and leaf drop. Sampling did not occur throughout the colder winter months (i.e. from June to September, 2014).

2.2.3.2. Monitoring the seasonal cycle of adults

The canopies of two blocks on each of the three farms were monitored fortnightly for the presence of adult moths. Yellow Delta Traps were lined with sticky pads (Chempac, Pty Ltd, Paarl, RSA) and baited with pheromone. Pheromone dispensers were synthesised in, and imported from, Lund University, Sweden (see Wang *et al*. 2015) on a regular basis to avoid the loss of pheromone efficacy (H. –L. Wang *pers. comm.*). Once imported, dispensers were stored at -20°C.

Due to the availability of pheromone dispensers, monitoring of adult male population abundances took place from September 2014 to September 2015.

2.2.4. Abiotic factors

Normal chemical spray programmes were followed in all blocks (see Appendix 2 for block names, plant dates, soil types and spray programmes of St Malo, Monte Cristo and Optenhorst).

Block-related factors such as altitude, aspect and size as well as structural aspects (of St Malo specifically), including trellis angle and light intensity, were recorded. In addition, weather data were obtained from the ARC – Institute of Soil, Climate and Water (AgroClimatology Staff. 2015. ARC-ISCW Climate Information System. ARC-Institute for Soil, Climate and Water, Pretoria).

2.2.5. Distribution

2.2.5.1. Current distribution

Of the five primary table grape producing regions in South Africa, three are located in the Western Cape. These are the Olifants River, the Berg River and the Hex River Valley. The course scale distribution of *H. capensis* throughout these regions was determined using pheromone traps (see above) and by physical observations of table grape and wine grape cultivars. Trapping was conducted at random in both table grape and wine grape vineyards, although in many cases, public access and limited communication with land owners restricted trap placements. Pheromone traps were left in vineyards for at least two weeks before collection.

In order to gauge the general distribution of the moth throughout the Western Cape, ad hoc sampling was conducted in areas surrounding the primary table grape producing regions. These areas included Stellenbosch, Grabouw and Hermanus. Pheromone trapping took place between January and March 2015.

In addition to the use of pheromone traps, any personal or public accounts of the moth were documented. In most cases, moths or larvae were sampled from these areas to confirm positive identification.

2.2.5.2. Historical distribution

During the winter months of 2014, *Rhoicissus* and *Cissus* herbarium samples from all over South Africa, stored in the Compton Herbarium, Kirstenbosch, were inspected. Samples indicating infestation symptoms (i.e. presence of mines, cocoon cavities adjacent to mines and cocoons) of any leafminers were photographed and noted. Date of collection as well as the area where the plant specimen was sampled, was recorded.

The geographical data resulting from trapping efforts and observations were plotted through the use of Google Earth (v7.1.2.2014, Landsat 2013).

2.2.6. Data analysis

2.2.6.1. Spatial trends associated with edge effects, block orientation and trellis systems

After testing the residuals from an ANOVA for normality, a non-parametric Mann-Whitney U Test was used in conjunction with a Generalised Non-Linear Model (GLM) with a log-link function to a Poisson distribution, since the residuals were not normally distributed, being discrete data. The GLM confirms significance in infestation incidents (defined as the abundance of leafmines and cocoons, mature abandoned leafmines were excluded to avoid pseudoreplication) between internal and external plots between all farms in both sampling seasons. This analysis was repeated for the two different trellis systems used (i.e. T-shaped trellis vs. Y-shaped trellis).

Analyses were done with restricted maximum likelihood (REML) in the VEPAC (Variation Estimation, Precision and Comparison) system of STATISTICA 12 to establish significance between the directionalities of block boundaries (edges).

2.2.6.2. Synchrony in infestation and damage symptoms

Due to the fact that infestation and damage symptoms (i.e. adult abundance, larval abundance, excavated mine abundance and cocoon abundance) can be linked to the different life stages of *Holocacista capensis* (at least, in part, to the duration of each life stage), lagged cross correlations (Chatfield, 1984), were performed between the damage symptoms

(abundances recorded on leaves and trellis posts) to verify observations made in the field (2014/2015 grapevine growing season) and in the laboratory.

Basic correlations (using correlation matrices) were conducted between larval and adult infestation, and all environmental variables (i.e. temperature, relative humidity and average rainfall) in the 2014/2015 grapevine growing season to establish trends in population fluctuations of the two life stages throughout a grapevine growing season.

2.2.6.3. Temporal patterns of the seasonal cycle of adults and the seasonal occurrence of larvae

All data collected regarding the abundance of larvae and adults were presented graphically to identify possible trends in abundance. Sampling methods in the 2014/2015 season were altered in order to discern between live and dead larvae. All immature larval mines that lacked any larval activity and absence of typical mature cocoon excavations were considered to be dead larvae.

The larval and adult abundance data (infestation per sample) for each fortnightly cycle was averaged and transformed logarithmically [log(x+1)], due to large numbers of zero counts. Resultant transformations were plotted against date, being the day of sampling. The male moth data were treated in a similar fashion. Correlation matrices were conducted on all abundance and weather data (also referred to as environmental variables).

All statistical analyses were conducted using STATISTICA 12 (Statsoft, Headquarters in Tulsa, Oklahoma, USA) and Microsoft Excel 10 (Microsoft, Headquarters in Redmond, Washington, USA). Unless otherwise stated, error bars denote the ± standard error of the mean.

2.3. Results

2.3.1. Ecology

Field and laboratory observations

In all cases, study sites were chosen based on the presence of any damage symptoms associated with *Holocacista capensis* (Appendix 1, Table 1.a). In many cases, infestation (in terms of damage symptom abundance) was far more extensive than considered by most growers. Concerns by growers were only raised if plant damage (leaves populated by leaf mines) was seemingly high and when cocoons were found attached to mature berries before harvest.

Whilst under observation in the laboratory, it was noted that feeding by larvae was less frequent under colder temperatures. Under warmer conditions, feeding by larvae was markedly rapid. Eggs, deposited within the epidermal cells of the leaf, could not be detected due to their microscopic size. Determining the larval instar of an individual proved to be difficult due to the fact that newly hatched larvae were virtually undetectable to the naked eye.

On one occasion moth infestation was noted on ground cover weeds growing adjacent to vines in de Heuvel. In another instance in a block of Dan Ben Hannah (Optenhorst) considerably high infestations were noted on shoots originating from Ramsey rootstocks (Appendix 1, Fig. 1.a).

Sex ratio

Little over 55% of the cocoons collected in the field and separated as male or female by overall dimensions of the cocoons were sexed correctly (as determined after eclosion). Of the eclosed cocoons collected in the 2014/2015 grapevine growing season the following male to female sex ratios were recorded: 30 March 2015 – 1 : 0.8 and 11 May 2015 – 1 : 1.1.

Differences in adult morphology

The most easily discernable differences in male and female morphology are differences in the wing and abdominal colouration (Appendix 1, Table 1.a). These can be observed using any hand-held magnifying glass as follows:

Wing pattern

The antemedial band of the forewing is continuous from the costal margin towards the hind wing in females (i.e. forms a solid white band over the wings) whilst in males this line is limited to being a small to medium-sized, broadly triangular, fleck on the dorsal part of the forewing. In some cases, the band is interrupted and may continue on the costal margin of the wing. The postmedial line of the forewing in females is almost as that in the wing of males, although somewhat more robust.

Abdomen

The terminal part of the male abdomen is pointed, whereas in the female it tends to be enlarged and squat. In addition, the abdominal segments in the male are silver/metallic in colouration, but the terminal segments are black in colour in the female.

Rearing temperatures in the laboratory

The results of the preliminary temperature experiments indicated that optimal rearing temperatures ranged between 20°C and 25°C where 100% of cocoons survived. Few mortalities occurred at 15 and 30°C (15% and 5%, respectively) whilst 100% mortality was experienced at 35°C. It should be noted, however, that pupae kept at 30°C eclosed before those held at cooler temperatures. Pupae kept at 15°C eclosed after those kept at warmer temperatures.

Lifecycle under controlled conditions

Of the live samples observed in the laboratory (those held at ambient temperature), the following durations were recorded for each of the life stages:

Day 1:	Oviposition (egg deposited within the leaf) on leaf margin
Day 4-5:	Small larval mines visible
Day 15-17:	Larva constructs cocoon casing
Day 27-29:	Adult moth emerges from cocoon
Day 31-35:	Mortality

Seasonal monitoring

Cross correlations (Fig. 2.2), indicated four week time lags between adult male moth abundance and live larval abundance ($r = 0.5364$) as well as between abandoned mature

mines and male moth abundance (r = 0.5952). As expected, correlations between full cocoons found on trellis posts and male moth abundance (r = 0.5685) were similar to that of abandoned mature mines and male moth abundance.

Overwintering strategy

The analysis of bark and leaf samples indicated a very low survival rate. Bark samples (a total of ca. 403 g) and leaf samples (a total of ca. 200 g) yielded 493 and nine cocoons, respectively. Of the cocoons originating from bark samples, only four adult moths were collected.

Parasitoids and predators

On three occasions, spiders and ants were found locating and removing new, suspended cocoons and cocoons rooted to trellis posts. The hymenopteran parasites collected included two species of eupelmids, an eulophid species, a mymarid species and two eurytomid species. Based on the relative abundances of the parasitoids emerging from both larval mines and pupae, it is unclear if parasitoids show any preference for a particular life stage. In the case of the eulophid and mymarid species, however, many of the samples were collected from cocoons and mines, respectively. An average of 3.7% parasitized mines were recorded, while an average of 11.5% parasitized cocoons were recorded in the 2014/2015 growing season over all farms.

Other leafminers/leafmining habits

Various larval and pupal samples were collected from the family Geraniaceae at various localities. A pupal sample (a cocoon) collected from *Rhoicissus tomentosa* (February 2015) residing in Suurbraak yielded a braconid wasp whilst eurytomids were collected from larval mines of *Holocacista varii* collected from *Pelargonium capitatum* (Kirstenbosch, April 2015) and a geranium (Montagu, May 2015).

2.3.1.1. Seasonal occurrence of larvae in relation to environmental factors

In the 2014/2015 grapevine growing season, larval infestation was recorded at least two weeks after bud break. The general trend observed throughout the two seasons included an increase in the mean abundance of larvae throughout the grapevine growing seasons (Fig. 2.3) of the vine (September onwards). In the 2013/2014 grapevine growing season (Fig. 2.3, A), the increase in larval abundance was much more gradual than that of the combined larval abundance recorded in the 2014/2015 grapevine growing season (Fig. 2.3, C). No significant correlations were recorded between the overall larval abundance and all environmental variables recorded (Table 2.1).

Larval abundances recorded in the 2014/2015 grapevine growing season indicate that live larval abundances fluctuate throughout the season and reach a peak in the first week of March (2015) while relatively high larval abundances were recorded as early as the beginning of November (2014) (Fig. 2.4). A marked decrease in live larval abundance was recorded in week 15 (mid-April). Throughout the season, beginning in September, the average abundance of dead larvae increased gradually until leaf senescence. No significant correlations ($p > 0.001$) were recorded between live- and dead-larval abundance, and all environmental variables recorded (Table 2.1).

Table 2.1: Correlations between overall-, live- and dead-larval abundances of *Holocacista capensis*, and the average maximum (max.) and minimum (min.) temperature (temp.), relative humidity (RH) and rainfall.

Larval abundance	**Environmental variable**	$\bar{x}$	***SD***	***r***	***p***
Overall*	Avg. max. temp.	29.1096	3.5934	0.0162	0.9524
	Avg. min. temp.	14.5995	2.6841	-0.1513	0.5759
	Avg. max. RH	80.1241	5.4986	-0.0803	0.7677
	Avg. min. RH	28.0819	6.3733	-0.1345	0.6193
	Avg. rainfall	0.3088	0.395	-0.1502	0.5788
Live	Avg. max. temp.	29.1096	3.5934	0.3517	0.1816
	Avg. min. temp.	14.5995	2.6841	0.3287	0.2138
	Avg. max. RH	80.1241	5.4986	-0.3418	0.1951
	Avg. min. RH	28.0819	6.3733	-0.3952	0.1298
	Avg. rainfall	0.3088	0.395	-0.0514	0.8502
Dead	Avg. max. temp.	29.1096	3.5934	-0.2449	0.3607
	Avg. min. temp.	14.5995	2.6841	-0.4636	0.0705
	Avg. max. RH	80.1241	5.4986	0.147	0.5869
	Avg. min. RH	28.0819	6.3733	0.1111	0.682
	Avg. rainfall	0.3088	0.395	-0.1727	0.5225

*Overall refers to both live and dead larvae.

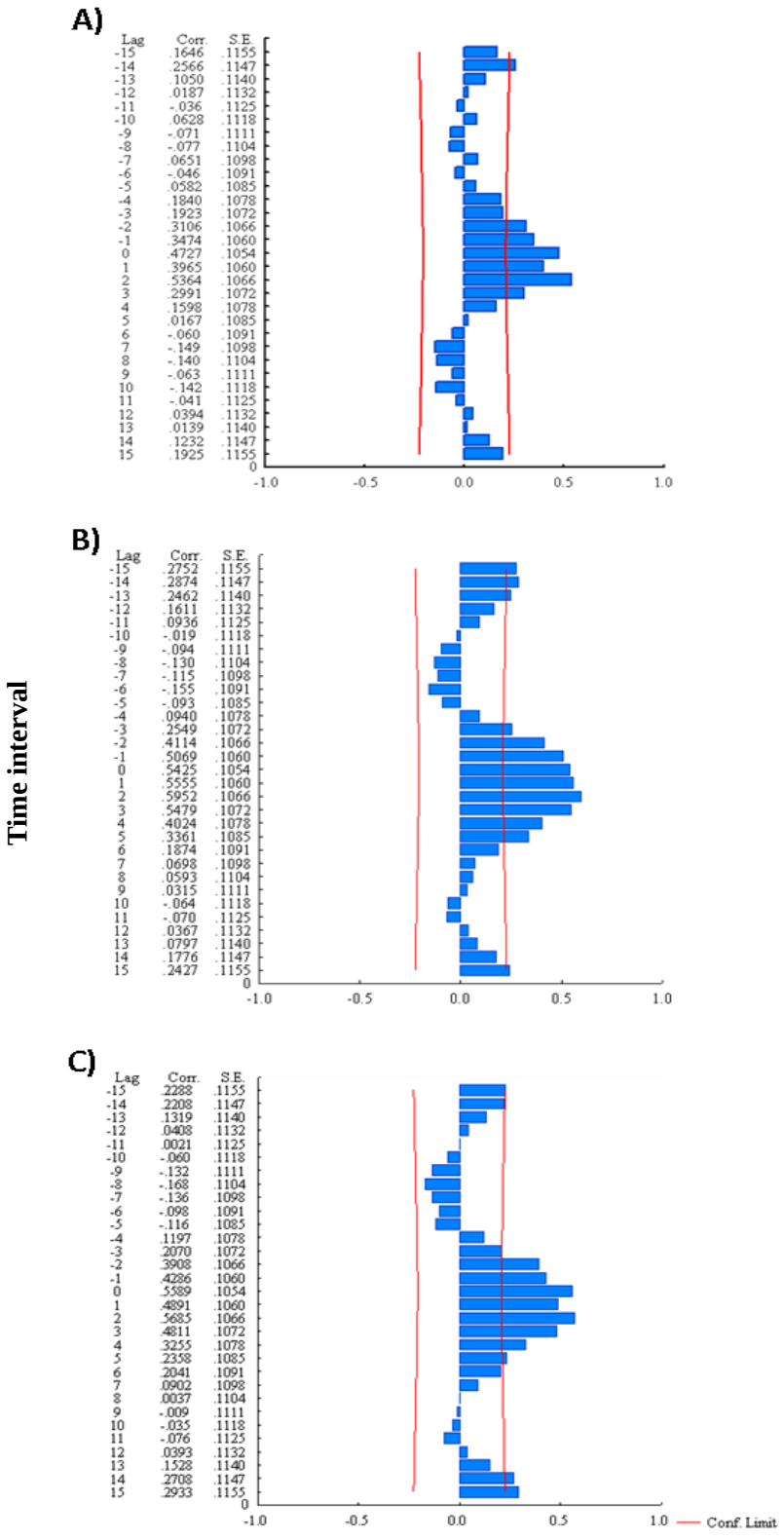

Figure 2.2: Cross correlations between infestation and damage symptoms (associated with *Holocacista capensis*) based on abundance data, collected throughout the 2014/2015 grapevine growing season. Cross correlations were conducted between adult male moths (first) and live larvae (lagged) (A), mature abandoned mines (first) and adult male moths (lagged) (B) and, full cocoons on trellis posts (first) and adult male moths (lagged) (C). One time interval = 2 weeks.

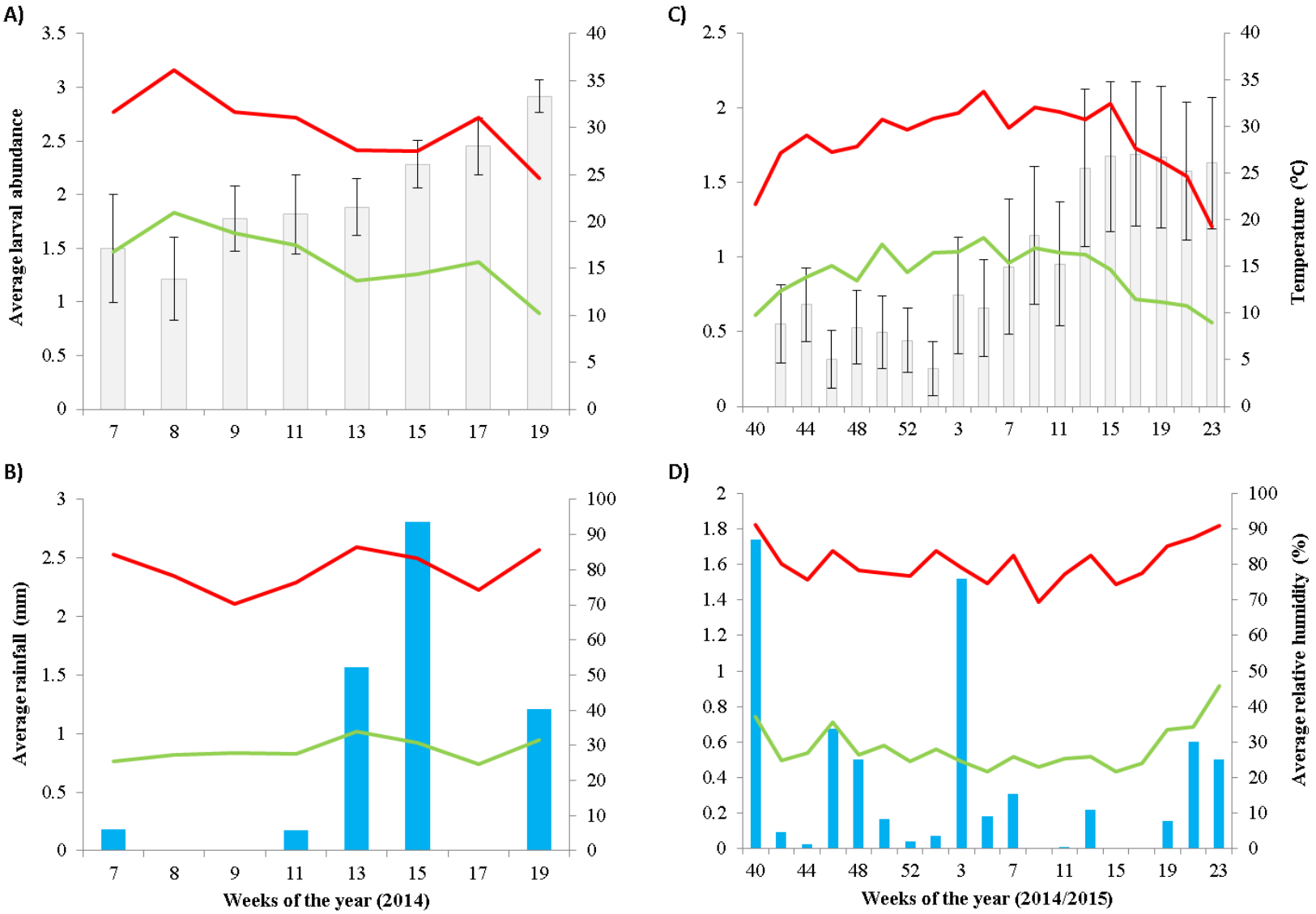

Figure 2.3: The seasonal occurrence of *Holocacista capensis* larvae (▭) (logarithmically transformed average abundance) (± S.E.) throughout the 2013/2014 grapevine growing season starting at week 7, 2014 (A) and throughout the 2014/2015 grapevine growing season starting at week 40, 2014 (C). The average maximum (—) and minimum (—) temperatures (°C) have been included in these figures. The average rainfall (mm) (▮) and average maximum (—) and minimum (—) relative humidity (%) have been included for both seasons (B and D, respectively). Week 1 = first week of January, 2015 (duration: weeks 1-4).

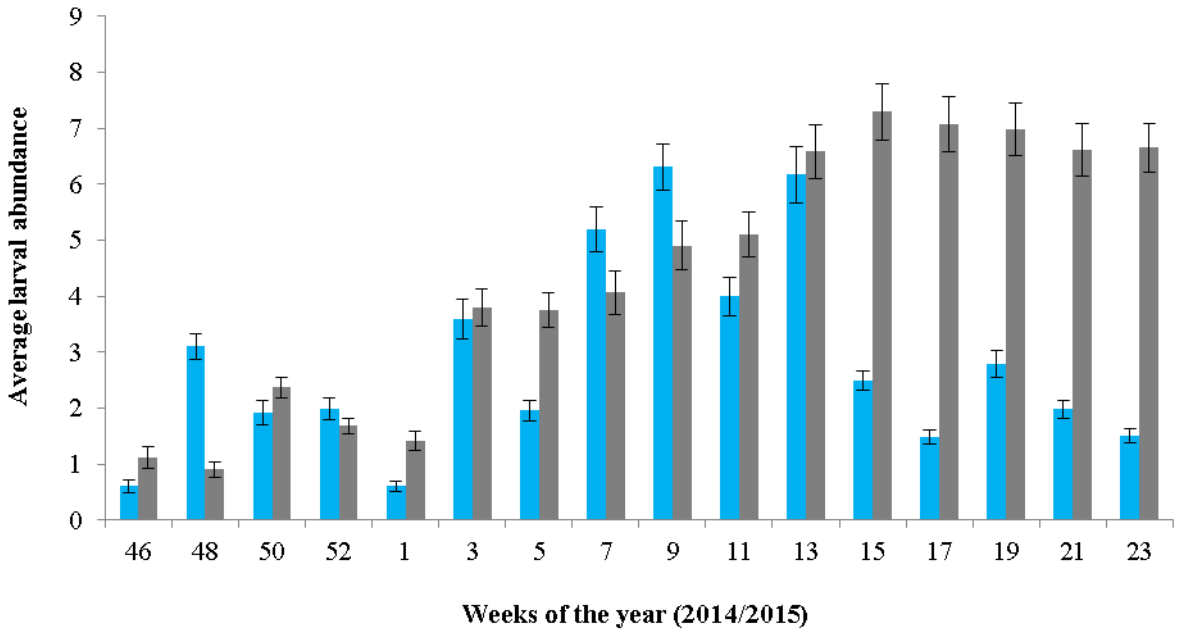

Figure 2.4: The average larval abundance (± S.E.) of live *Holocacista capensis* larvae ( ) and empty immature mines ( ) recorded in infested leaves throughout the 2014/2015 grapevine growing season. Week 1 = first week of January, 2015 (duration: weeks 1-4).

2.3.1.2. Seasonal cycle of adults

Throughout the 2014/2015 grapevine growing season, pheromone traps indicated several fluctuations in the adult male moth population (Fig. 2.5). When trapping began in early September (week 38), adult abundances were relatively high. Peaks in population abundances were experienced in the middle of November (week 46), the end of December (week 52), the beginning of February (week 5), the end of March (week 13) and again in the beginning of September (week 37). In the beginning of the season clear declines in the adult male population were recorded (i.e. mid-October and end November 2014). Thereafter, considerable declines were only recorded from the end of May.

The average adult abundance was most strongly correlated with the average minimum relative humidity ($\bar{x}$ = 28.0819; SD = 6.3733; r = -0.6556; p = 0.0058), but was also significantly correlated to the average maximum temperature ($\bar{x}$ = 29.1096; SD = 3.5934; r = 0.5684; p = 0.0216). No significant correlations were recorded between the abundance of adult males and live ($\bar{x}$ = 2.8561; SD = 1.7806; r = 0.4606; p = 0.0725) or dead ($\bar{x}$ = 4.3945; SD = 2.3389; r = 0.384; p = 0.142) larvae.

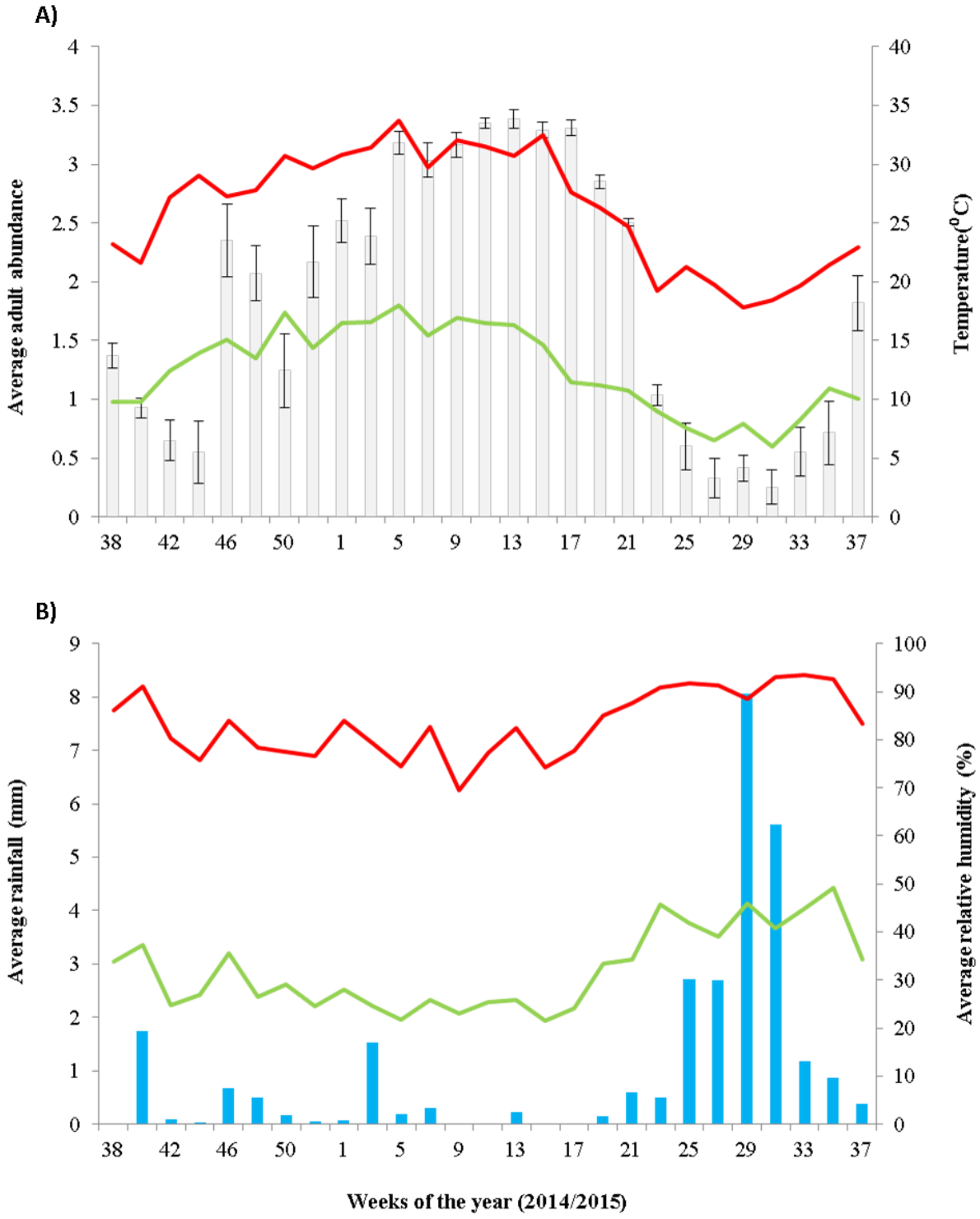

Figure 2.5: The seasonal cycle of adult male *Holocacista capensis* (moths) (▭) (logarithmically transformed average abundance) (± S.E.) throughout the 2014/2015 grapevine growing season (A). The average maximum (—) and minimum (—) temperatures (°C) have been included in these figures. The average rainfall (mm) (▬) and average maximum (—) and minimum (—) relative humidity (%) have been included (B). Week 1 = first week of January, 2015 (duration: weeks 1-4).

2.3.1.3. Edge effects

In the 2013/2014 grapevine growing season infestation was significantly higher in internal plots than in external plots (Wald $X^2_{(1)} = 47.952$; $p < 0.001$; df = 1) (Fig. 2.6) whilst in the 2014/2015 grapevine growing season no significant results were observed between internally and externally located plots (Wald $X^2_{(1)} = 1.0313$; $p = 0.30984$; df = 1). Further analyses confirmed that the latter result was not attributed to a decrease in overall infestation (Optenhorst analysed independently) (Wald $X^2_{(1)} = 0.563$; $p = 0.4529$; df = 1) or early season monitoring efforts (only sampling months February to May 2015 included in the analyses) (Wald $X^2_{(1)} = 0.2052$; $p = 0.6506$; df = 1).

No significance was recorded between the edge orientated aspects for all blocks in the 2013/2014 grapevine growing season ($F = 0.5792$; $p = 0.6371$; df = 3) and the 2014/2015 grapevine growing season ($F = 0.6832$; $p = 0.5792$; df = 3).

2.3.1.4. Trellis systems

Infestation incidents differed significantly between trellis systems. Infestation incidents in Y-shaped trellis systems was significantly higher than that of the T-shaped trellis systems in the 2013/2014 grapevine growing season (Wald $X^2_{(1)} = 269.242$; $p < 0.001$; df = 1) and the 2014/2015 grapevine growing season (Wald $X^2_{(1)} = 58.6551$; $p < 0.001$; df = 1) (Fig. 2.7).

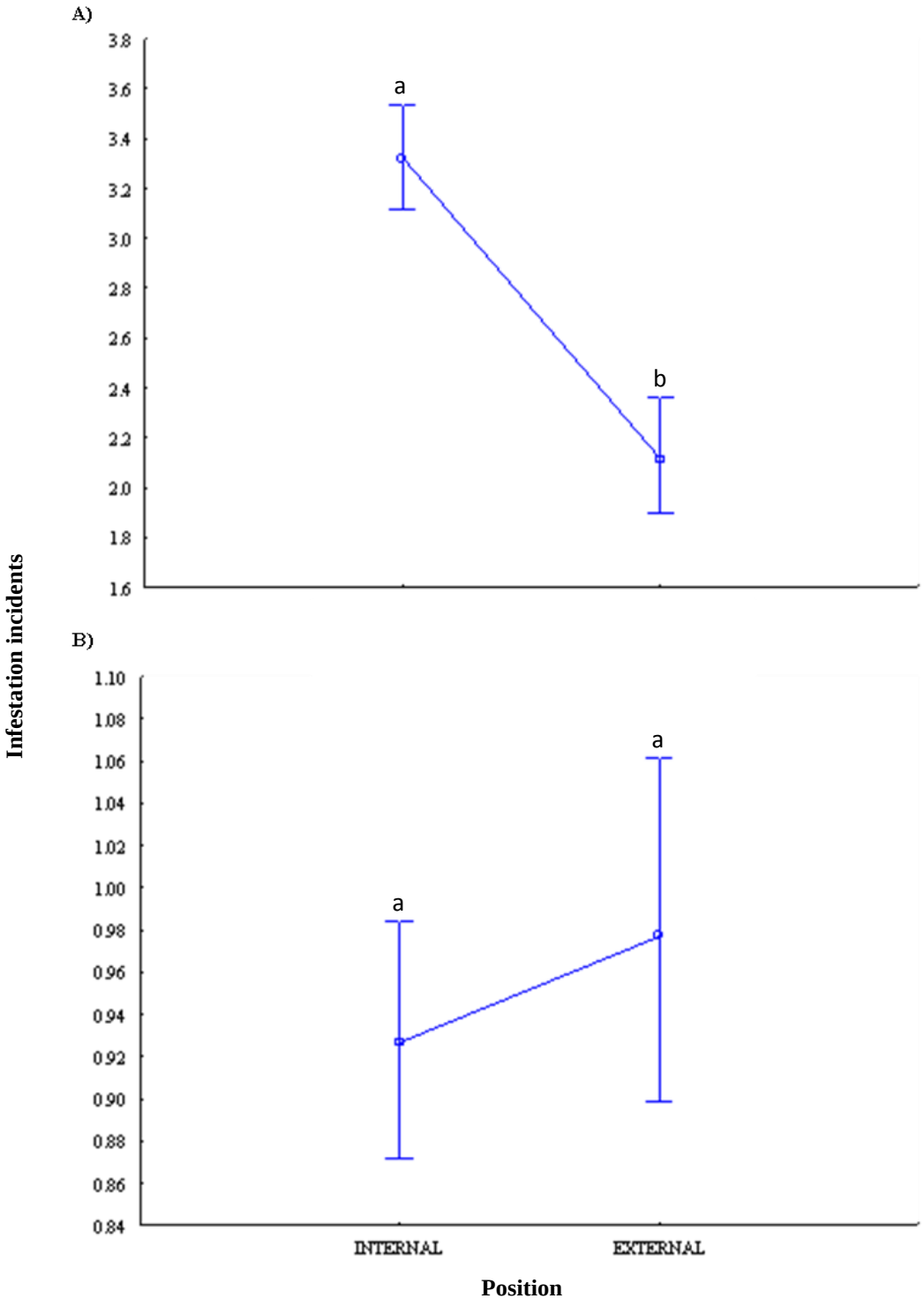

Figure 2.6: Generalized Non-Linear Models obtained from the analysis of infestation incidents of *Holocacista capensis* in internally and externally located plots in the 2013/2014 (A) and 2014/2015 (B) grapevine growing seasons. A deviation in lower case lettering indicates significance in plot location within a grapevine growing season. Vertical bars denote 0.95 confidence intervals.

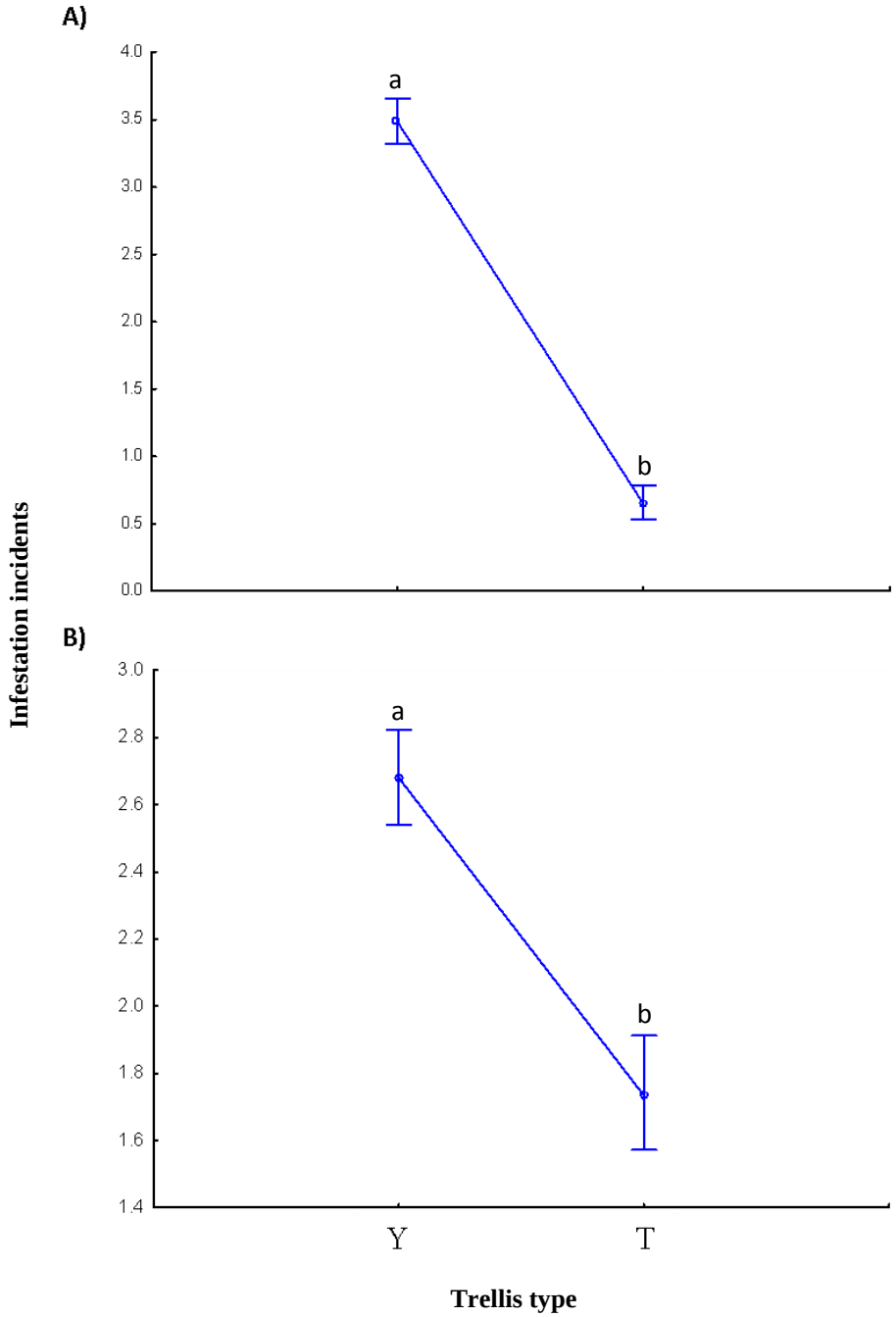

Figure 2.7: Generalized Non-Linear Models obtained from the analysis of infestation incidents of *Holocacista capensis* in blocks characterized by different trellis systems (Y-shaped and T-shaped) in the 2013/2014 (A) and 2014/2015 (B) grapevine growing seasons. A deviation in lower case lettering indicates significance between trellis types within a grapevine growing season. Vertical bars denote 0.95 confidence intervals.

2.3.2. Distribution

2.3.2.1. Current distribution

Trapping efforts indicated that *Holocacista capenis* is widespread throughout the Western Cape. Moths were detected in all traps placed throughout the Berg River region (Fig. 2.8; Appendix 3). An average of ca. 770 adult male moths were caught per trap per fortnight. Adult male moth abundance was considerably high in Riebeeck Kasteel (3492 male moths trapped), Tulbagh (2192 male moths trapped) and Piketberg (1787 male moths trapped). All other trap catches were limited to a total catch (per town) of less than 300 male moths. In September of 2014, eclosed cocoon remnants (bark samples) were collected from a small vineyard in Philadelphia.

Fairly low populations were detected in the Hex River Valley (Fig. 2.9). An average of two adult male moths were caught per trap. The highest abundance of moths was recorded near McGregor (15 male moths trapped). Of the eight traps placed in De Doorns (Appendix 3), two traps were placed a few kilometres before entering the De Doorns region located before the table grape producing hub (Fig. 2.9). A total of four adult male moths were collected from these two traps. No moths were detected within De Doorns. In April of 2014, larval samples were collected in Red Stone Hills (situated between Oudtshoorn and Calitzdorp).

Ad hoc sampling was carried out in Stellenbosch, Suurbraak, Elgin, Hermanus and the Olifants River. Infestation was only reported in Vredendal (see Appendix 3), Stellenbosch and Hermanus (only three adult moths detected).

General observations

Although fairly widespread within the Western Cape, growers outside of Paarl and Wellington were not aware of *Holocacista capensis* despite the high infestations recorded in Riebeeck Kasteel, Tulbagh, Piketberg and Vredendal. In the case of Optenhorst and St Malo, it was only when cocoons were found on mature grape bunches that concerns were raised.

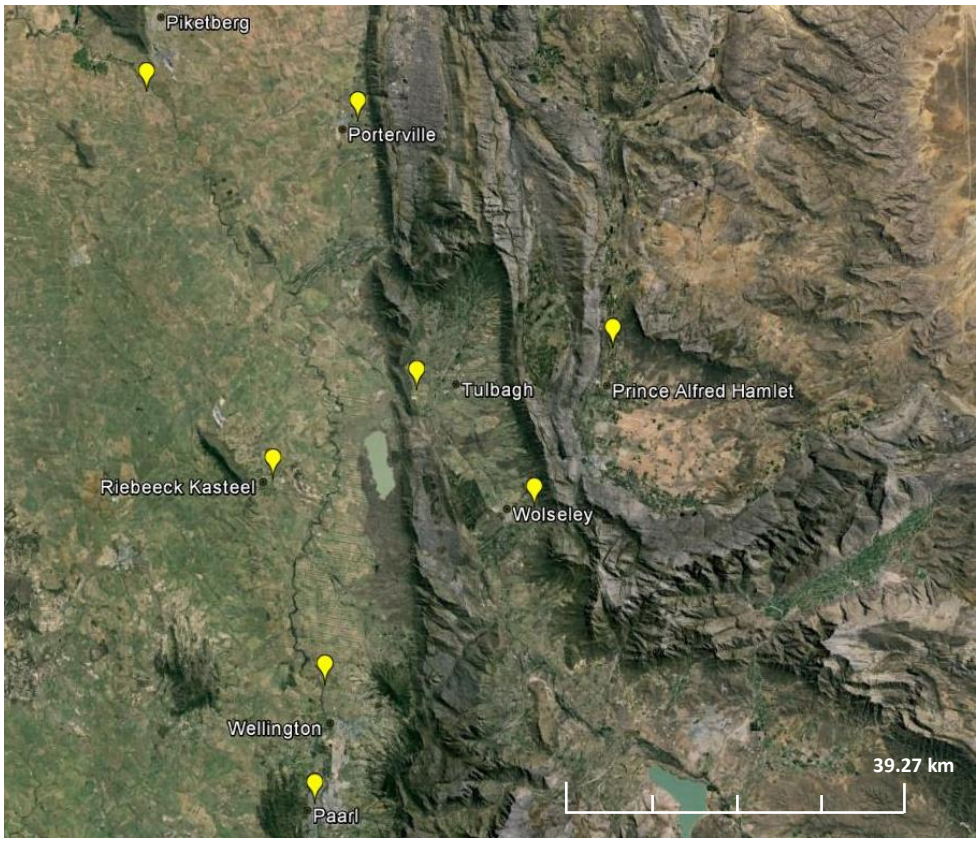

Figure 2.8: The distribution of *Holocacista capensis* throughout the Berg River, Western Cape.

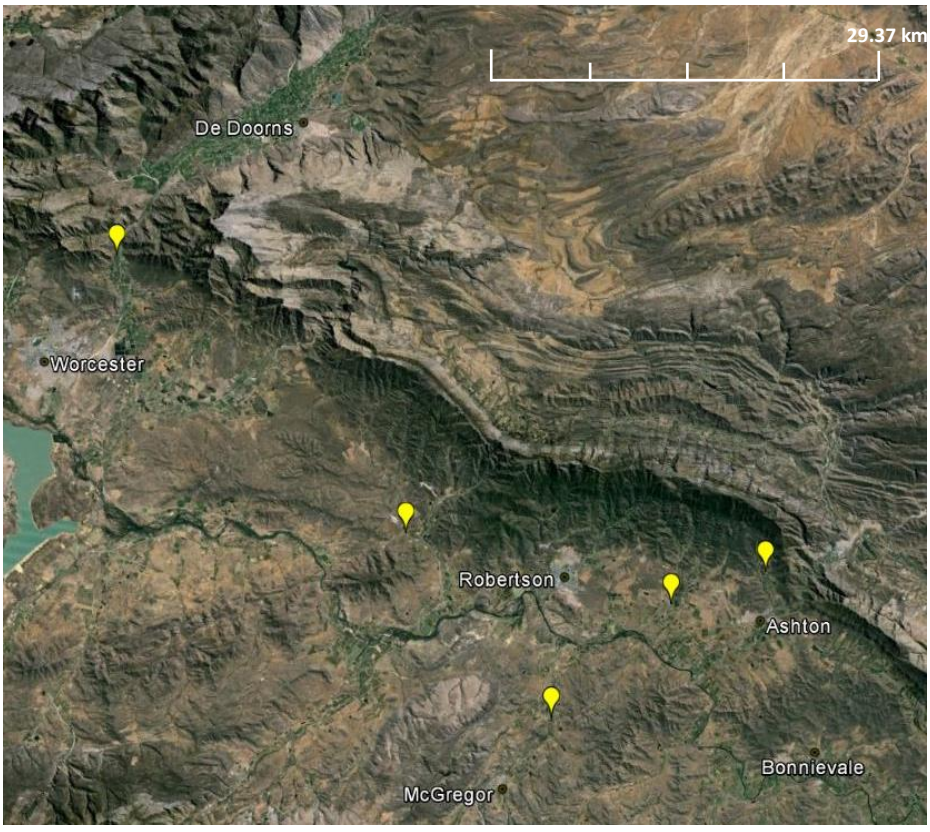

Figure 2.9: The distribution of *Holocacista capensis* throughout the Hex River Valley, Western Cape.

2.3.2.2. Compton Herbarium

A variety of leafmines were observed on dried *Rhoicissus* and *Cissus* samples housed in the Compton Herbarium (Appendix 4). Many of the samples originated from coastal regions along the East and West Coasts of South Africa. Leafmines similar to those of *Holocacista capensis* were found predominantly on *R. digitata* and *R. tridentata* ranging from the West Coast to Swaziland and Limpopo.

The localities of leafmines detected in herbarium samples are presented in Figure 2.10.

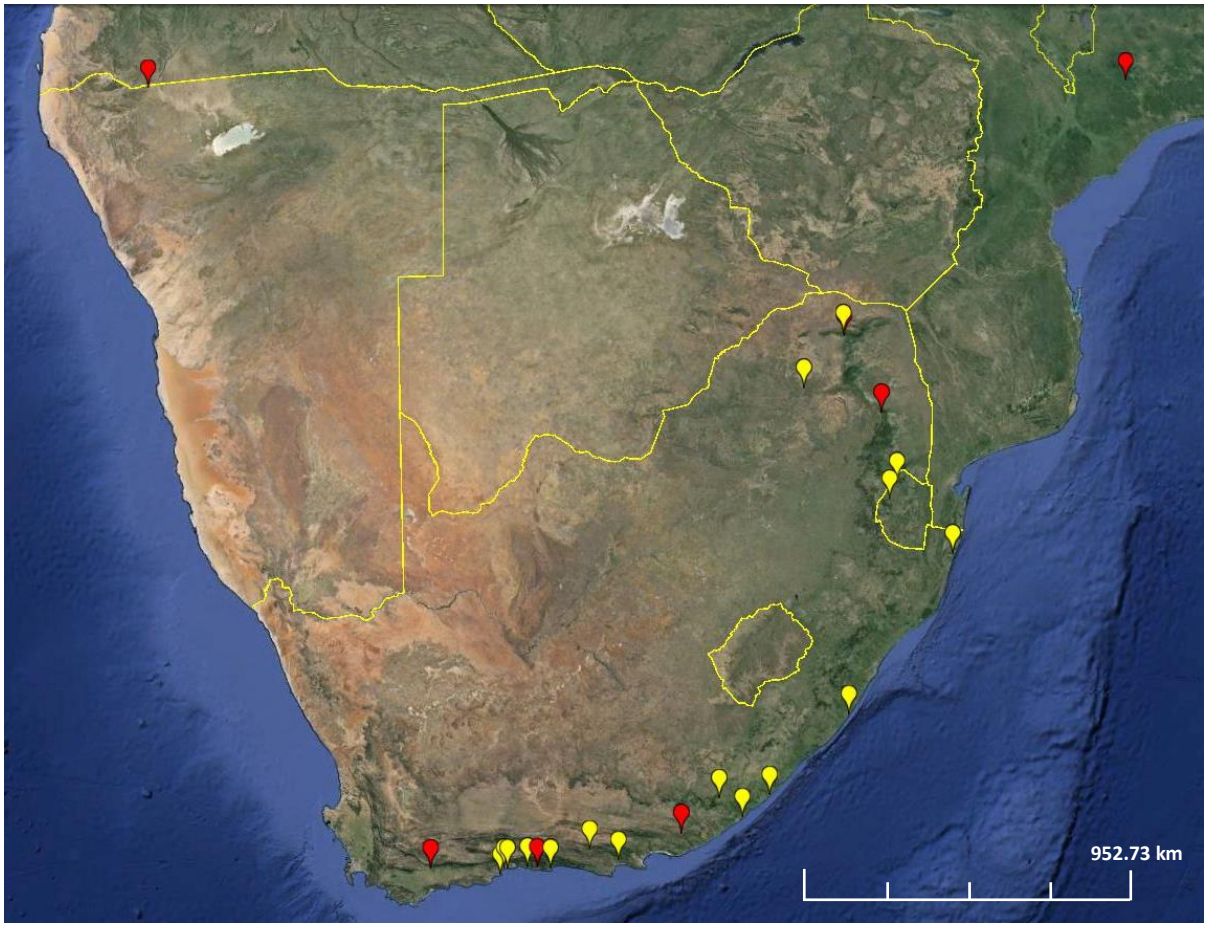

Figure 2.10: A geographical depiction of leafminer infested *Rhoicissus* and *Cissus* samples (see Appendix 4) analysed in the Compton Herbarium (Cape Town, South Africa). Red indicators indicate a presence of leafminer whose mines are dissimilar to that of *Holocacista capensis* whilst yellow indicators are representative of samples that harboured mines similar to that of *H. capensis*.

2.4. Discussion

Mazanec (1984) explored the use of larval gonads of *Perthida glyphopa* Common (Lepidoptera, Incurvariidae) as a sexing tool to explain unequal sex ratios observed in adults. In addition, differences in the morphology of the last abdominal segments of male and female pupae of *Phyllocnistis citrella* and *Cameraria ohridella* Deschka & Dimic (Lepidoptera: Gracillariidae) have been established by Jacas & Garrido (1996) and Freise & Heitland (1999), respectively. In the case of *H. capensis*, it is theorised that in general, larger, more irregularly shaped cocoons contain females whilst smaller more elliptical cocoons contain males (H. Geertsema *pers. comm.*).

Sexing based on cocoon morphology was unreliable whereas adult morphology and wing pattern differentiation proved to be a reliable means of differentiating between male and female moths. The sex ratios recorded from field-collected cocoons in 2014 were largely inconclusive, however, it is highly likely that the male and female sex ratios fluctuate throughout a season and there may be periods where one sex is in higher abundance than the other. A variety of factors could influence such fluctuations (Hardy 1997). Future studies should be aimed at establishing differences between male and female larvae and pupae. Sexing such individuals would aid in the establishment of the sex ratio of local populations earlier in the season when suspended cocoons are not in high abundance. To determine accurate sex ratios throughout the different generations or progenies, studies should be focused on establishing the larval instar that any given larva is in at the time of sexing (in the event that several overlapping generations are present in a population at any given time). Through the determination of these factors, valuable insights regarding the rate of larval mortality and the effect of sex ratio on that statistic (if any) may be gathered.

Pilot trials aimed at establishing the optimal rearing temperature of the pupae of *H. capensis* suggest that the optimal rearing temperature ranges are between 20°C and 30°C. The average temperature recorded for weeks 3 to 13 (based on the presence of live larvae recorded in the field), in Paarl and Wellington, is approximately 24°C which supports this assumption. The mortality recorded at 30°C (one individual) could be attributed to parasitism, miscellaneous death or improper handling of cocoons when collected, however, great care was taken to reduce damage to cocoons collected in the field. The parasitoids collected from field-

collected larval mines and cocoons should be studied further in order to determine their potential as biological control agents.

Considering the fact that the lifespan of adult moths is less than a week, the recorded seasonal fluctuations of adult male moth populations are indicative of multivoltine behaviour as speculated in Nieukerken & Geertsema (2015). Seasonal changes in life-history events for these insects in temperate zones are common (McGregor 1997). Due to the fact that adult populations correlate strongly with temperature and live larval abundances do not, it is clear that several overlapping generations are present at an infested farm at any stage throughout the productive stages of the grapevine growing season. In June 2015, an increase in temperature was followed by an increase in adult abundance which suggests that pupae have the ability to undergo diapause (as discussed previously). In the event that only the larvae undergo diapause, prior to pupation, fluctuations in adult abundance would not be as responsive to temperature fluctuations. Pereira *et al*. (2007) found that weather conditions, natural enemies and plant quality attributes were the main determinants of the population dynamics of *Leucoptera coffeella* Guerin-Meneville (Lepidoptera: Lyonetiidae) which could explain the differences observed and recorded in the laboratory and in the field, respectively.

At room temperature the life cycle of the moth was completed in less than five weeks, whereas the time lags recorded under variable environmental conditions suggest that the life cycle of the moth in the field takes more than seven weeks to complete (that is if the egg to fully matured larval stage is conservatively estimated as three weeks in duration). The variability in environmental factors is most likely the largest contributing factor to the major discrepancies between laboratory and field related observations and time lags recorded. The controlled conditions of the laboratory can be related to even conditions or at least, less variable conditions than those experienced in the field.

Leafmining activity recorded on leaf samples suggest a gradual increase in leafminer infestation throughout the season. When live larval abundance was separated from dead larval abundance in the 2014/2015 grapevine growing season, it became apparent that there was a high level of larval mortality that occurred throughout the season, leading to a supposed “build-up” of larval activity (based on overall leaf damage). When differentiating between the live and dead larvae it became clear that the larval stage also experiences considerable population fluctuations. The mechanisms behind these fluctuations are not clear

and could be caused by a combination of cyclic seasonal conditions (the larval abundances seem to fluctuate regularly every six to eight weeks), environmental factors and parasitism.

In the 2014/2015 grapevine growing season, live larval populations reached a maximum abundance from the middle of February to the end of March, whereas adult populations reached a peak from the beginning of February to the end of April. This phenomenon is the main contributing factor to the infestation intensity in successive seasons and is probably caused by the eclosion of cocoons produced earlier in the season that exploit structural aspects (e.g. attaching themselves to the underside of bark and small crevices in trellis posts) which enables them to escape predation by parasitoids and predators, despite the fact that microclimate conditions may not be favourable for minimised development time. The decrease in live larval abundance could be attributed to a corresponding increase in parasitoid communities, further encouraged by the decreased use of pesticides before harvest.

Fenoglio *et al*. (2009) reported a higher percentage of mined patches and leafminer abundance of *Liriomyza commelinae* (Frost) (Diptera: Agromyzidae) on *Commelina erecta* L. (Commelinaceae) in more urbanized areas. This could be true for the blocks situated in Optenhorst (in the centre of Paarl). In the 2012/2013 grapevine growing season, H. Geertsema (*pers. comm.*) recorded a high abundance of *H. capensis* (cocoons and adults), the intensity of which was only experienced again in the 2014/2015 growing season. The decrease in abundance of *H. capensis* recorded in 2014 could have been a result of the spray programme adopted in the previous growing season (i.e. due to the use of dichlorvos).

Generally, it is the case for most heliozelids that the larvae overwinter inside the cocoon throughout the colder winter months (Stehr 1992). It is believed that the larvae only pupate a few weeks prior to emergence (Stehr 1992). To date, no information or hypotheses regarding diapause or the overwintering strategy of *H. capensis* have been gathered or considered and it is not yet known if the larvae or pupae overwinter as in the case of pupae of *Phyllonorycter ringoniella* (Matsumura) (Lepidoptera: Gracillariidae) (Li *et al*. 2002). Contradictory to studies on the overwintering strategy of *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) conducted by Lim & Hoy (2006), the pilot trials conducted in the current study suggest that larvae enclosed in cocoon casings (prior to pupation) and undergo diapause in the colder winter months. However, there is also the possibility that moths overwinter in adjacent weedy ground cover in the agricultural landscape based on observations made at de Heuvel

during 2014. The former theory is strongly supported by the observations of the colony exposed to winter conditions. The presence of live larvae in cocoons produced in the autumn months of 2014 confirms that at least the larvae do undergo diapause.

In the case of *L. coffeella*, *Phytomyza chaerophylli* (Kaltenbach) and *Agromyza frontella* (Rondani) (Diptera, Agromyzidae), temperature and photoperiod have been identified as important cues for diapause regulation (Tauber *et al*. 1982; Frey 1991; Nestel *et al*. 1994). Winter diapause is considered to be maintained by low temperatures and short photoperiod. It has been found that temperature can override the effect of photoperiod in winter and thus temperature seems to be the most important signal for winter hibernation maintenance and termination (Tauber *et al*. 1982; Frey 1991). A benefit of this is that late-season generations may be produced if pupae experience favourable temperature conditions for growth and development later in the season (Tauber *et al*. 1982). The malleable diapause described above could only ever evolve in species that experience short life cycles, further evidence that multiple generations of *H. capensis* are present in a single season. Similarly, Frey (1991) found that the diapause experienced by *P. chaerophylli* provides a high degree of flexibility in its life cycle which allows close adaptation to the phenology of specific host plant populations.

Warmer climates are known to facilitate more generations (Tauber *et al*. 1982) which compliment laboratory observations, but does not shed light on the relationship between larval abundance and temperature. As a result, the effect of microclimates on the developmental rates and pupae may play an extremely important role in seasonal variations in larval and adult abundances as large variation may be experienced within a relatively small area (Tauber *et al*. 1982; Li *et al*. 2002). Studies by Chen & Kang (2004) found that some *Liriomyza huidobrensis* Blanchard (Diptera: Agromyzidae), found beyond the northern distribution border in China, do not show enhanced cold tolerance, and that those that do survive in severe winter conditions survive only by opportunistic exploitation of protected microhabitats. Fluctuations in microclimatic conditions could explain the presence of *H. capensis* adults throughout the winter.

Severe leafminer infestation was treated with dichlorvos in the 2013/2014 and 2014/2015 grapevine growing seasons on all farms (Appendix 2). In all cases, dichlorvos was used as a final attempt to decrease leafminer abundance before spray regimes were arrested, prior to

harvest (A. Carstens; H. Carstens; R. King *pers. comm.*). A major downfall to this strategy is that the higher leafminer abundance experienced later in the season is not addressed, allowing pupae to accumulate after harvest (many of which are likely to overwinter and contribute to infestation experienced in the following season). Pre-emptive applications of spinetoram began in October in St Malo, a farm that suffered severe infestation the 2013/2014 grapevine growing season. These applications were applied to severely infested Red Globe and surrounding blocks. Leafminer infestation in the following season (the 2014/2015 grapevine growing season) was markedly low. Chemical control measures are discussed in greater detail in Chapter 4. The effect of pesticides on the natural enemies could also contribute to an increase in host population.

Infestation incidents (i.e. the presence of leafmines) were significantly higher in blocks whose vines were trained along a Y-shaped trellis system as compared to those of a T-shaped trellis system. This finding could be as a result of varying microclimates formed by the availability of direct sunlight and the resultant effect on temperature within the microclimate as discussed above. From observations made in the field, Y-shaped trellis systems facilitated the penetration of more direct sunlight compared to those of T-shaped (or roof-like) trellis systems. Similar to findings of Nielsen & Ejlersen (1977), Basset (1991) suggested that general leafmining activity on *Argyrodendron actinophyllum* (Bailey) Edlin, 1935 appeared to be higher on more shaded and larger leaves in contradiction with the findings mentioned above. Basset (1991) recorded significantly higher leafminer incidents in the lower leaf canopy (lower light intensity). Future studies should be aimed at determining the effect of light intensity and leaf size on *H. capensis* abundance as this may shed light on oviposition and dispersal behaviour. In addition, the findings of the present study should be studied for a longer period over several more blocks and farms in the future. In the 2013/2014 grapevine growing season high infestations were recorded on Red Globe in St Malo where no T-shaped trellis systems were used. Similarly, it was Optenhorst that suffered high infestations in the following season where only one of the three blocks tested was of a T-shaped trellising system (Crimson). Other factors (e.g. cultivar, spray programme and prior infestations) may be confounding the results pertaining to significance between trellis systems.

Blackburn *et al*. (1999) stated that individual species (almost without exception) exhibit great spatial variation within the local densities which they attain, and a species may be entirely absent from most places, at relatively high densities in a small proportion of occupied sites

and at relatively low densities in the majority of the places where they occur. A study by Cornelissen & Stiling (2009) on the abundance and survivorship of the leaf-mining moth *Cameraria* sp. nova throughout the range of its host plant, *Quercus myrtifolia* (Fagaceae), observed higher abundances at the edges of the distribution and found that the abundance of mines and mine survivorship (more specifically successful emergence) was similar between range sites in close proximity to each other. It is suggested that the latter (directly affected by natural enemies) might contribute to variation in *Cameraria* densities over the range, however, the variation among sites does not show a distinct spatial structure and plant quality seemed to be an important determinant of spatial distribution.

Several studies have been conducted on the spatial distribution of species and the deleterious effects of edge effects. Generally, spatially associated species abundance is coupled to environmental gradients (Sagarin & Gaines 2002). Range edges are, in many cases, subject to water and/or nutrient deficits that affect both the physiology and quality of host plants. When the spatial distribution of *H.capensis* is considered, it could be possible that leafmining larvae benefit from elevated nitrogen arising from intermittent water stress (see Münster-Swendsen 1984). Species such as these have the ability to avoid compartmentalized allelochemicals, causing higher insect herbivore infestation on stressed plants at the range edge (Larsson 1989). If this is the case, it is likely that the deficit of rain in the 2014/2015 grapevine growing season led to widespread infestation of the leafminer within a grapevine producing block.

The interaction and varied responses by natural enemies (one of the three mechanisms underlying edge-associated differences in the patterns and prevalence of stage-specific survivorship) of a leafmining Dipteran was noted as an important factor in the spatial distribution of individuals throughout any given area (McGeoch & Gaston 2000). Bowman *et al*. (2002) found that species that do not exhibit strong edge effects should not have high emigration rates, immigration being the dominant process. As it is not clear whether or not *H. capensis* exhibits strong edge effects and the effect of patch size (i.e. farm/block size) on infestation incidents is not known, no clear conclusions can be made regarding their immigration and emigration behaviour (i.e. dispersal). It may be important to consider the impact of area (i.e. block size or farm size/isolation) on infestation incidents in future studies.

The distribution of *H. capensis* throughout the Western Cape is far more widespread than previously thought. Interestingly, infestation was higher in areas west of the Drakenstein mountain range (i.e. Paarl, Wellington, Riebeeck Kasteel, Tulbagh and Piketberg), as compared to any other areas. Infestation prevalence in these areas may be result of higher temperatures (lower altitudes) and environmental conditions characteristic of the region (e.g. strong southerly winds). Infestation was present within the Hex River Valley, however, infestation levels were very low and leaf damage was scant when field visits ensued. No infestation was recorded within De Doorns, the table grape producing hub of the Hex River Valley. Despite the fact that overall infestation in the Hex River Valley is low, monitoring efforts should be adopted to monitor infestation presence in De Doorns specifically. Preventative measures can be taken if individuals are detected before bud break (see Chapters 3 and 4). Relatively high adult populations were detected in Vredendal, but traps placed in areas in close proximity to the site where moths were detected did not yield any moths (e.g. Klaver and Trawal). This implies that infestation can be localized and infestation throughout an area can go undetected (e.g. in De Doorns) based on the location of a trap relative to the presence of moths. To establish a finer distribution of infestation in the Western Cape, trapping efforts should be extended. To confirm the identification of *H. capensis*, DNA barcoding should be conducted in future studies.

Infestations noted on *Rhoicissus* and *Cissus* samples housed at the Compton Herbarium, Kirstenbosch, support the prediction of Cape origins of *H. capensis* made by Nieukerken & Geertsema (2015). Although species identity cannot be confirmed, it is highly likely that populations in the Western Cape originated from the forests and coastal thicket of the Sunshine Coast. It is likely that ornamental vines and grapevine scions distributed by nurseries have facilitated the dispersal of *H. capensis* throughout southern Africa as discussed by Nieukerken & Geertsema (2015). To confirm predictions such as these, surveys of moth presence in naturally occurring stands of *Rhoicissus* and *Cissus* should be conducted with the aid of pheromone traps. Future studies should consider the importance of host plant shifts in the evolution of phytophages and should aim to determine the prevalence of Vitaceae in the host record of the Heliozelidae. *Cameraria ohridella* Deschka & Dimic (Lepidoptera, Gracillariidae), an invasive horse chestnut leafminer, has been found to colonise highly populated stands of horse chestnut before colonising the countryside, a phenomenon typical of stratified dispersal where dispersal is affected by both long-distance movements and local diffusion (Gilbert *et al*. 2004).

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Chapter 3

Monitoring and action threshold determination of the Cape grapevine leafminer (*Holocacista capensis*)

3.1. Introduction

The management of all economically important leafminers has been largely limited to the application of synthetic chemical insecticides (e.g. abamectin and cyromazine; see Chapter 4) (Stein & Parrella 1985; Weintraub & Horowitz 1997) which contribute to the loss of beneficial insects, the development of insecticide resistance, the resurgence and establishment of primary and secondary pests, and the exposure of harmful chemical compounds to humans and the environment (Stein & Parrella 1985).

In South Africa, the high prevalence of a newly described leafmining lepidopteran, *Holocacista capensis*, in table grape vineyards in the Berg River of the Western Cape (Chapter 2) has raised concerns of growers and the table grape industry alike. Since its first discovery in 2012, the control of *H. capensis* has only been attempted through the inadvertent application of dichlorvos in the grapevine growing season when infestation has progressed to cocoon-infested berry bunches (A. Carstens; H. Carstens *pers. comm.*). In these cases, added labour costs are incurred due to the fact that cocoons must be manually removed from the berry bunches after harvest. This is achieved using small brushes and delicate handling to dislodge cocoons attached to a berry's surface and to avoid blemishing the bloom of the grapes, respectively (H. Carstens *pers. comm.*). The latter is especially important for red cultivar varieties (H. Geertsema *pers. comm.*).

Damage symptoms by this leafminer include the presence of small to medium-sized foliar blotch mines (mature mines usually ca. 15 mm in length) and the presence of their small cocoons attached to grapevine berry bunches, vine foliage, stems and to trellises. Mature, abandoned mines are characterized by a perfectly elliptical cavity on the margin of a yellowing leafmine. In most cases, management is only considered later in the season when leaf damage is noticeable (i.e. after an accumulation of mature, abandoned, empty and parasitized mines), as a result of superficial foliar inspections earlier in a grapevine growing season. Adult leafminer abundance may, however, become considerably high from as early as

early November (Chapter 2) in the first few months of a grapevine growing season. By this stage the berry bunches of late season cultivars are often contaminated with cocoons (as indicated in Chapter 2). In addition, there are legislative restrictions on the presence of chemical residues (by the use of insecticides) on berry bunches intended for export (de Villiers & Pringle 2007), inhibiting the ability to apply insecticides in abundance and too close to harvest. The development and use of a monitoring system can, however, improve pest detection, making it possible to avoid the inappropriate administration of chemical sprays (Brown & Pringle 2006).

It is clear that management strategies need to be adopted earlier in the season either through preventative measures or infestation based strategies in order to reduce the implementation of unnecessary chemical applications. The problem arises, however, when infestation is variable throughout the seasons, as the fundamental principles of Integrated Pest Management (IPM) strategies state that the use of insecticides should only be applied when needed (Kogan 1998). To effectively exercise such strategies, a species-specific monitoring program should be established to determine the presence and density of *H. capensis* to further direct management thereof (Walton *et al*. 2004). More specifically, monitoring strategies are used to develop economic thresholds which serve to reduce costs associated with pest control and improve crop quality by aiding in decision making within the multifaceted approach (see Kogan 1998), associated with IPM strategies (Weddle *et al*. 2009). When considering any monitoring strategy, it is vital that the information obtained from it is reliable, as the findings thereof will be used for making critical management decisions (de Villiers & Pringle 2007).

The objective of this study was to establish an action threshold, based on *H. capensis* adult male moth abundance (by the use of pheromone-baited traps) and grapevine bunch infestation. This would be the first step towards developing a robust management strategy against *Holocacista capensis*.

3.2. Methods and materials

3.2.1. Study sites

This study was conducted on each of the three farms (and corresponding blocks) as described in Chapter 2 (2.2.1).

3.2.2. Monitoring adult male moths using pheromone traps

Adult male population fluctuations were monitored on a fortnightly basis using yellow Delta Traps that were lined with sticky pads (Chempac, Pty Ltd, Paarl, RSA) and baited with pheromone as explained in Chapter 2 (2.2.3.2). Monitoring was conducted from September 2014 to September 2015.

3.2.3. Monitoring leaf- and bunch-infestation

The sampling methods of de Villiers & Pringle (2008) and Walton (2003) were adapted to conduct grapevine inspections (see Chapter 2 sections 2.2.1 and 2.2.3.1) as follows: Within each block, 20 evenly distributed plots (internal plots), each consisting of five vines, were chosen along five equally distributed rows. An additional plot was established at the end of each row (external plots) where an additional 10 leaves were collected per row. Visual monitoring was used to analyse bunch infestation within the internally located plots. One berry bunch on each of the five vines within a plot was inspected using a presence/absence sampling system. In addition to using fruit infestation and adult abundance as factors indicative of infestation, damage symptoms on leaves were also considered by inspecting one leaf from each of the five vines within each plot (described in Chapter 2, section 2.2.3.1). Each block was sampled fortnightly.

Monitoring was carried out over a period of 15 months, from February, 2014 to May, 2015. During the two grapevine growing seasons, leaves were sampled (to monitor fluctuations in larval populations) until leaf senescence and leaf drop. Sampling did not occur throughout the colder winter months (i.e. from June to September, 2014).

3.2.3.1. Defining infestation variables

In order to establish the damage symptom that is best associated with physical moth counts, correlations (using correlation matrices) were carried out between both cocoon- and mine-infestation and adult male moth abundance. Lagged cross correlations (Chatfield, 1984) were further conducted to determine how well damage symptoms were synchronised with adult abundance. The correlations were repeated on the adjusted data to confirm the findings of the former. Mature, abandoned mines were not considered as an appropriate damage symptom indicative of infestation as older, mined leaf parts could be mistaken for other leaf damage [e.g. bacterial blight (*Xylophilus ampelinus*)] or leaf senescence.

3.2.4. Data analysis

3.2.4.1. Pheromone trap catch vs. bunch infestation

3.2.4.1.1. Weighted regression analyses

The adult moth abundance from pheromone traps recorded in each of the blocks was transformed to the natural log (ln) whilst the cumulative infested bunches were transformed to empirical logits (Cox & Snell 1989) for each of the sampling dates. Empirical logits were regressed on the natural log of the trap counts using a weighted regression with the reciprocal of the variance of the empirical logits as the weighting co-efficient (Cox & Snell 1989). Empirical logit transformation makes use of a 0.5 adjustment factor, which addresses granularity in the data (Mirman 2014). The empirical logit was calculated using:

$$Z_j = \ln\left(\frac{R_j + {}^1/_2}{n_j - R_j + {}^1/_2}\right), j = 1, 2, 3 \ldots N \text{ samples} \qquad (1),$$

and the variance (Cox & Snell 1989),

$$V_j = \frac{(n_j+1)(n_j+2)}{n_j(R_j+1)(n_j-R_j+1)}, j = 1, 2, 3 \ldots N \text{ samples} \qquad (2),$$

where Z_j is the empirical logit for the *j*th sample, R_j is the number of infested bunches in the *j*th sample, n_j is the total number of bunches in the *j*th sample and V_j is the variance of the empirical logit for the *j*th sample.

The weighted regression was calculated using:

$$Logit(z) = a + (b)\ln(n) \quad (3),$$

where Logit(*z*) are the empirical weighted logits, *n* is the average number of adult male moths caught in the pheromone-baited traps and, *a* and *b* are regression constants.

The empirical weighted logits, Logit(z), were back-transformed using expression 4. This expression could, therefore, be used to estimate the proportion of infested bunches for any number, *n*, of males trapped (Cox & Snell 1989).

$$z = \frac{\exp[a+b(n)]}{1+\exp[a+b(n)]} \quad (4)$$

Expression 4 was solved for *n* by iteration using *z* = 0.05 (5% bunch infestation). As no economic thresholds have been determined for *H. capensis*, it was assumed that an infestation level of 5% bunch infestation was acceptable based on assumptions made by de Villiers & Pringle (2007) regarding other grapevine pests.

3.2.4.2. Bunch infestation between farms and between cultivars

Dummy variable regression (Gujarati 1970a; Gujarati 1970b; Draper & Smith 1998) was used to describe differences in bunch infestation between different farms and cultivars respectively. These analyses were conducted to assess potential bias within the results.

All statistical analyses were conducted using STATISTICA 12 (Statsoft, Headquarters in Tulsa, Oklahoma, USA) and Microsoft Excel 10 (Microsoft, Headquarters in Redmond, Washington, USA). Unless otherwise stated, error bars denote the ± standard error of the mean.

3.3. Results

3.3.1. Monitoring leafminer infestation

General trends in the severity of infestation (typical damage symptoms used as an indication thereof), recorded throughout the 2013/2014 and 2014/2015 grapevine growing seasons, varied considerably (Fig. 3.1). For both mature, abandoned mines and larval mines, abundances were much higher in the 2013/2014 grapevine growing season than in the 2014/2015 grapevine growing season. The number of cocoons recorded on leaves in the 2014/2015 was much higher than that recorded in the 2013/2014 grapevine growing season (Fig. 3.2). The average number of infested leaves recorded throughout the 2013/2014 and the 2014/2015 grapevine growing seasons indicate that the former season experienced more severe leafminer infestations.

Throughout the 2014/2015 grapevine growing season the infestation of cocoons on trellis posts in Optenhorst (the farm with the highest observed overall infestation in the 2014/2015 grapevine growing season), indicated a low level of parasitism when compared to full, undamaged cocoons (Fig. 3.3). Similar to leafminer and adult abundance, parasitism also increased as the season progressed (Fig. 3.3).

3.3.2. Lagged cross correlations between adult abundance and leaf infestation

Preliminary correlation matrices indicated that cocoon and larval abundance on leaves were significantly correlated with adult male moth abundance ($r = 0.5688$; $p < 0.001$; $n = 106$ and $r = 0.4951$; $p < 0.001$; $n = 106$, respectively). A two and four week time lag was recorded between adult abundance and cocoon infestation ($r = 0.6116$) and between adult abundance and larval infestation ($r = 0.6413$), respectively (Fig. 3.5).

3.3.3. Correlation between adult abundance and bunch infestation

A significant, positive relationship between bunch infestation and adult moth abundance was prevalent in the 2014/2015 grapevine growing season ($F_{(1, 47)} = 46.81$, $r^2 = 0.499$, $p < 0.001$)

(Fig. 3.6). The relationships seem more defined for infestations within Optenhorst (Fig. 3.6, A). Within Optenhorst, the relationships are clear (clustered groupings evident) between Alphonse Lavallée and Dan Ben Hannah (Fig. 3.6, B). The progressive bunch infestation and adult male moth infestation observed throughout the 2014/2015 grapevine growing season (Fig. 3.4) support these findings.

3.3.4. Bunch infestation between farms and between cultivars

Significant differences in bunch infestation were recorded between farms ($F_{(2, 157)} = 18.676$, $p < 0.001$) and between cultivars ($F_{(7, 152)} = 26.141$, $p < 0.001$). Bunch infestation was highest in Optenhorst ($p < 0.001$), whilst no significant differences in bunch infestation were recorded between Monte Cristo and St Malo ($p = 0.9801$). Throughout the 2014/2015 grapevine growing season, bunch infestation was highest in Dan Ben Hannah ($p < 0.001$) than on any other cultivar. No significant differences were recorded between the other cultivars.

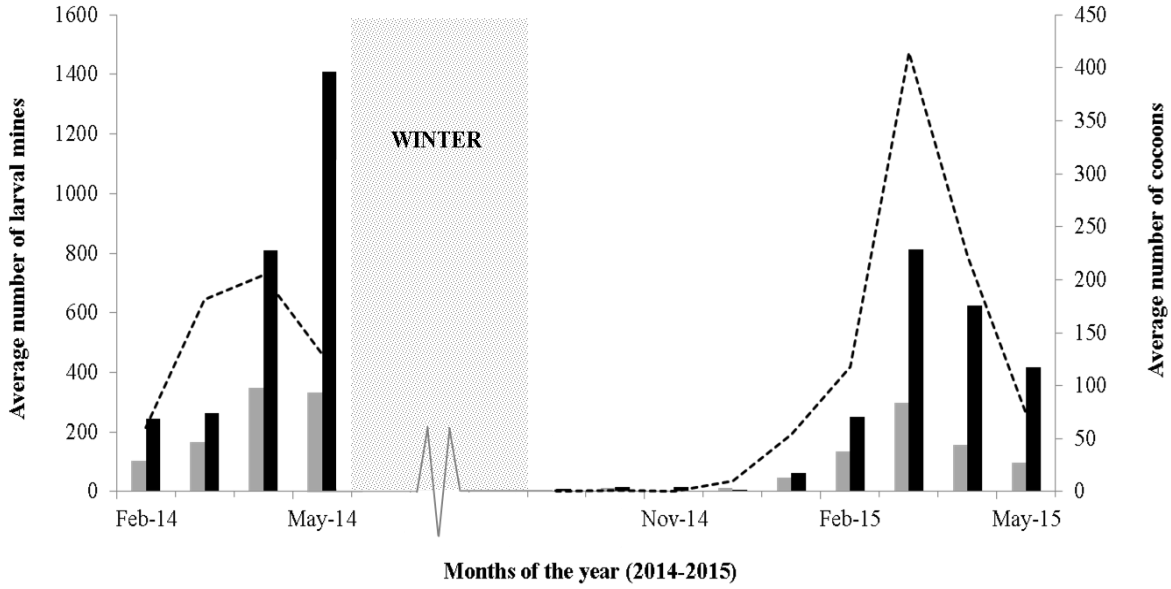

Figure 3.1: The average number of *Holocacista capensis* larval mines (▬); mature, abandoned mines (▬) and cocoons (- - -) recorded on leaves, on all farms, throughout the 2013/2014 and 2014/2015 grapevine growing seasons. The 2014/2015 grapevine growing season ended in May 2014 and the 2014/2015 grapevine growing season started in September 2014. During winter (grey shaded area), no monitoring took place.

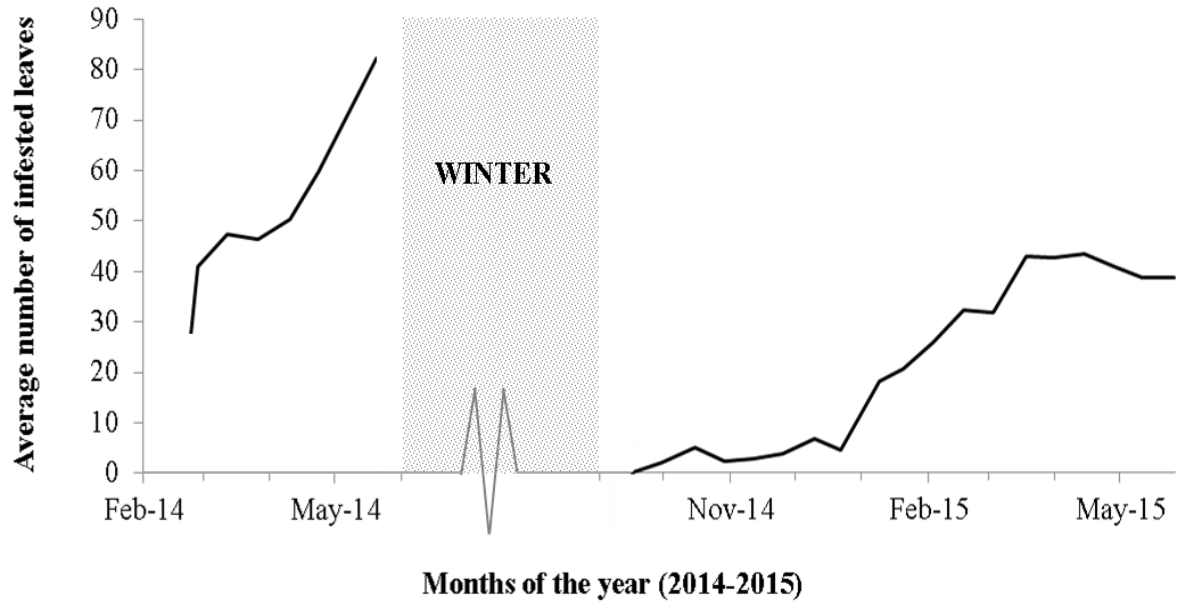

Figure 3.2: The average number of leaves infested (150 leaves/block) by *Holocacista capensis* throughout the 2013/2014 and the 2014/2015 grapevine growing seasons. The 2013/2014 grapevine growing season ended in May 2014 and the 2014/2015 grapevine growing season started in September 2014. During winter (grey shaded area) no monitoring took place.

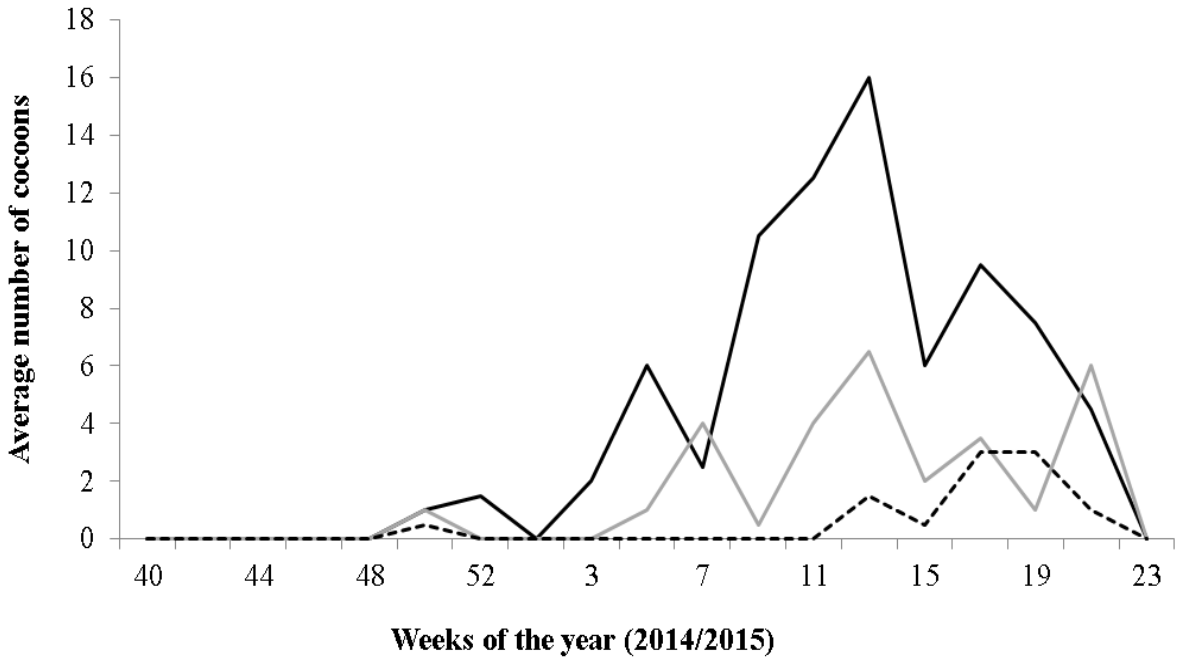

Figure 3.3: Average number of full *Holocacista capensis* cocoons (▬), empty or damaged cocoons (▬) and parasitized cocoons (▬ ▬) recorded on trellis posts in Optenhorst throughout the 2014/2015 grapevine growing season. Week 1 is the first week of January (duration: week 1-4).

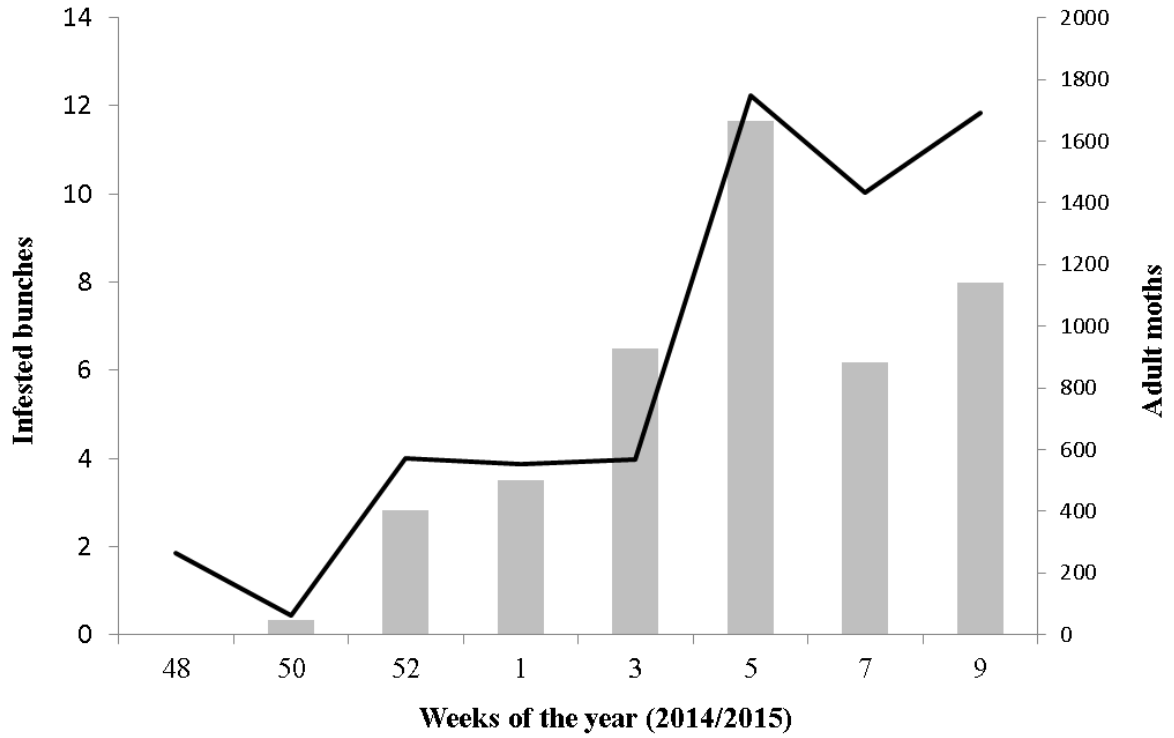

Figure 3.4: The average number of bunches infested by *Holocacista capensis* cocoons (▬) and the average number of adult male moths (—) counted and collected from all farms/fortnight throughout the 2014/2015 grapevine growing season. Week 1 = first week of January, 2015 (duration: week 1-4).

3.3.5. Action threshold associated with adult male moth abundance and bunch infestation

The significant, positive relationship between bunch infestation and adult moth abundance described in section 3.3.3. can be represented by the following regression equation:

$$Logit(z) = -9.12 + (1.1014)\ln(n)$$

Solving for *n*, in expression 4, at an economic threshold of 5% bunch infestation (z = 0.05) (Fig. 3.7) yielded an action threshold of 442 male moths per trap per fortnight. However, in cases where *H. capensis* is considered a phytosanitary risk (export grapes), a 0% bunch infestation should be considered (z = 0.00). One percent bunch infestation was recorded when 87 adult male moths were present.

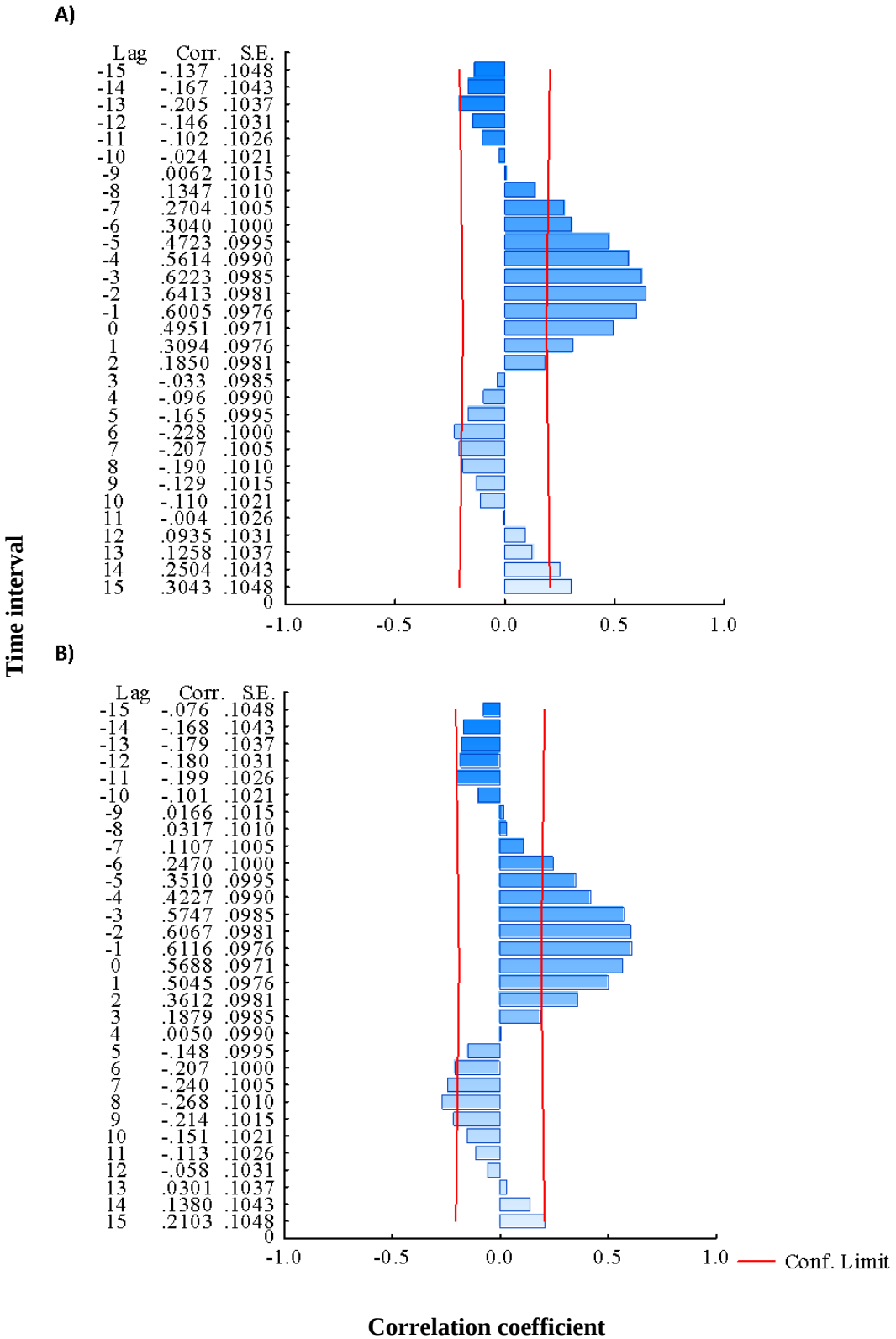

Figure 3.5: Cross correlations between leaf infestation and damage symptoms and the emergence of adult male *Holocacista capensis* (moths) based on abundance data collected throughout the 2014/2015 grapevine growing season. Cross correlations were conducted between all adult male moths (first) and all larvae (lagged) (A) and between all adult male moths (first) and all cocoons (lagged) (B). One time interval = 2 weeks.

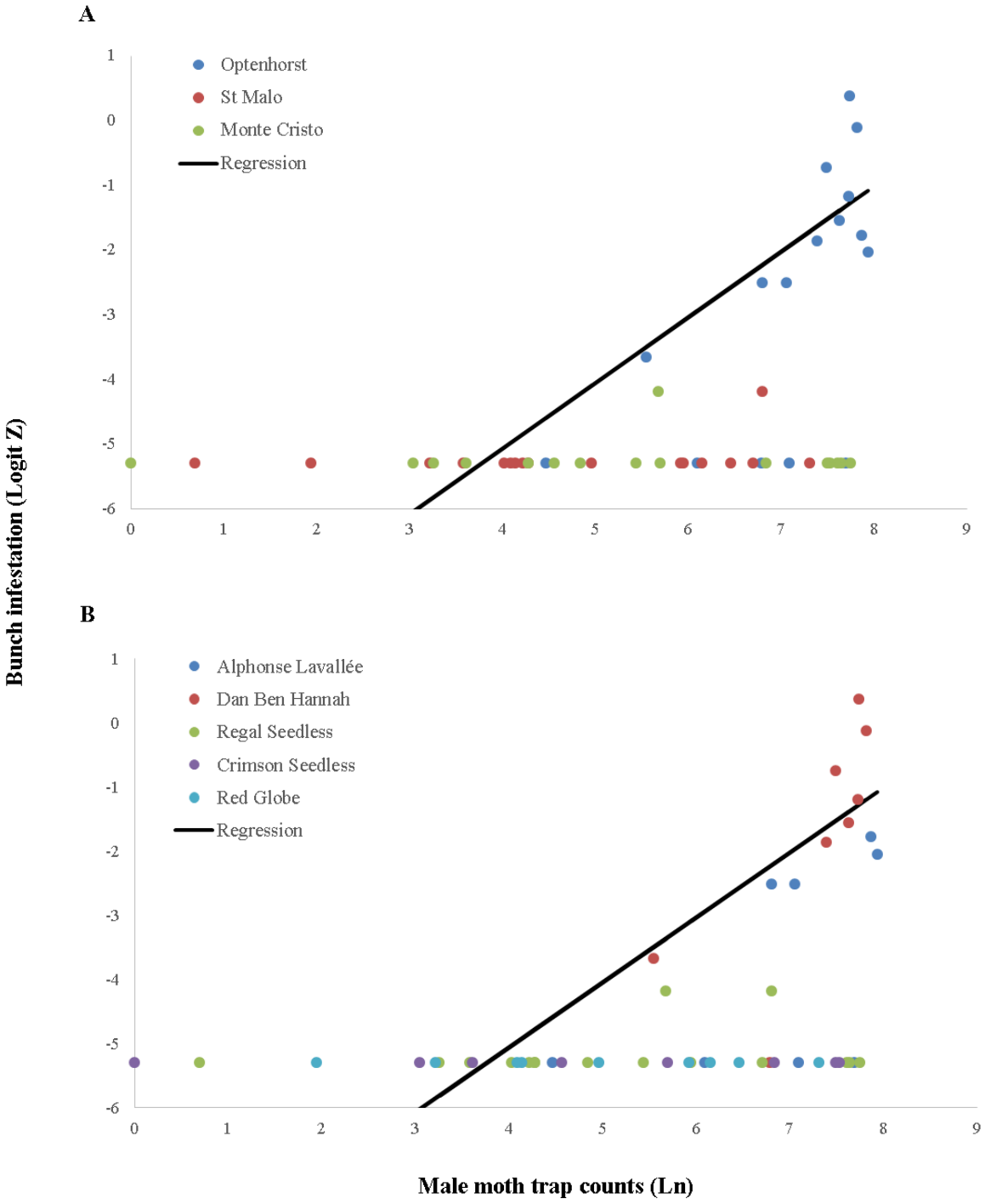

Figure 3.6: Weighted regression of Logit (z) on Ln (n) where z is the proportion of infested fruit and n is the number of adult male *Holocacista capensis* (moths) caught per trap per fortnight between farms (A) and between cultivars (B).

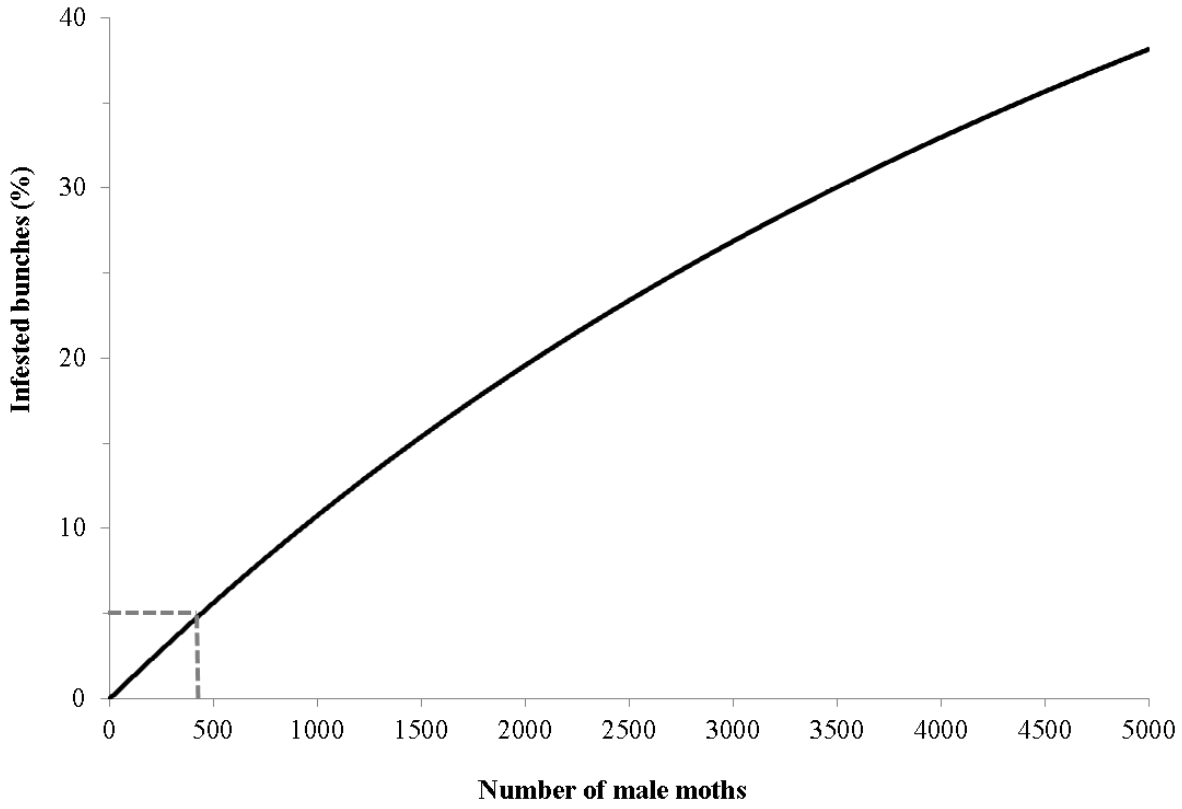

Figure 3.7: The relationship between infested bunches (%) and the total number of adult male *Holocacista capensis* (moths) caught within a block/fortnight after back transformation from empirical logits. The interrupted lines indicate the estimated adult male population density at which 5% of bunches are infested (**---**).

3.4. Discussion

Monitoring efforts have indicated that leafminer infestations are variable between seasons and often vary between farms and cultivars. It is clear, however, that there is a progressive accumulation of leafminer infestation and damage symptoms throughout a growing season. This is especially important when monitoring systems are considered for monitoring pests. A progressive accumulation of infestation may indicate that the variability associated with decisions to apply management strategies may decrease as a grapevine growing season progresses. Interestingly, trends in larval and cocoon abundances indicated lower infestations in the 2014/2015 and 2013/2014 grapevine growing seasons, respectively. The increased presence of cocoons in the 2014/2015 grapevine growing season suggests, however, an increase in the survival rate of larvae. This phenomenon could be linked to changes in leafminer/predator/parasitoid dynamics (potentially affected by the use of chemical applications intended for the control of other grapevine pests) or a reduction in average relative humidity in the 2014/2015 grapevine growing season.

Correlations recorded between adult male moth infestation and leaf infestation suggest that cocoon infestation could be used to develop a monitoring system. A monitoring strategy could be established based on methodologies set out by Binns *et al*. (2000), Madden *et al*. (1996), Madden & Hughes (1999), Reusink & Kogan (1994), Taylor (1961), Davis (1994), Suits (1957), Gujarati (1970a, 1970b) and Draper & Smith (1998). It should be noted, however, that the use of both cocoon and larval leaf infestation, as appropriate indicators of infestation, may not always be reliable indicators of infestation (and thus may not always be reliable traits to consider for decisions regarding management intervention) based on unclear trends in their seasonal occurrence and seasonal cycle, respectively. In addition, it is highly likely that bunch infestation is a possibility as soon as infestation progresses to leaves infested with cocoons. A monitoring system based on leaf-infested cocoons should, therefore, only be considered in cases where 5% bunch infestation, as suggested by de Villiers & Pringle (2007), is tolerated. In addition, leaf sampling is laborious due to the fact that the monitoring thereof involves the documentation of actual cocoon counts on leaves and the inspection of both the adaxial and abaxial surfaces of a leaf. As a result, additional labour costs may be incurred when several blocks are to be inspected.

De Villiers & Pringle (2007) emphasise the need to develop a monitoring system that is sensitive enough to detect the lowest possible pest prevalence, as even low levels of fruit infestations may lead to the rejection of consignments. Due to the fact that a significant positive relationship was recorded between bunch infestation and adult male moth abundance, and that adult male moths can be detected throughout the year (even under colder winter conditions, see Chapter 2), the monitoring of adults at the start of a grapevine growing season would provide the earliest and most reliable indicator of potential bunch- and leaf-infestation.

Results indicate that an economic threshold of 5% bunch infestation an action threshold of 442 male moths caught per trap per fortnight is yielded, whilst 1% bunch infestation was achieved when 87 adult male moths were present. This finding suggests that pheromone traps could be used as an early screening tool before leaf monitoring protocols are conducted, in addition to providing valuable action threshold values.

It is important to consider that the differences noted between seasons, farms and cultivars demonstrate differences in *H. capensis* densities temporally and among blocks (i.e. areas or

regions) which will inevitably impact the relationship between fruit infestation and adult male trap counts. The development of a reliable monitoring system should be conducted over several grapevine growing seasons and on several more farms and cultivars distributed throughout the Western Cape to obtain a more robust monitoring system, which incorporates known margins of error for bunch- and leaf-infestation.

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Chapter 4

An investigation of potential management strategies and postharvest management protocols, focused on the control of the Cape grapevine leafminer *(Holocacista capensis)*

4.1. Introduction

The Cape grapevine leafminer, recently described as *Holocacista capensis* Nieukerken & Geertsema (2015), has been identified as a pest of quarantine importance on table grapes in the Western Cape. Recent studies conducted on the identification of the pest by Nieukerken & Geertsema (2015) and the biology of the pest (Chapter 2) have indicated that the control of *H. capensis* may be troublesome due to its multivoltine behaviour and increased population abundance experienced in the later months of a grapevine growing season (in many cases after harvest).

In the past two growing seasons, only chemical and physical measures were taken to reduce moth populations noted in the early-season. These measures included the application of dichlorvos and spinetoram prior to harvest, as well as the manual removal of cocoons from berry bunches after harvest. In most cases, mid-season chemical applications are restricted by withholding periods related to the use of any chemical product (used to ensure that residues on treated produce will not exceed the maximum residue limit) on all fruit intended for export (APVMA 2014).

During harvest, grapes are placed in lugs which are stored in a precooling facility as soon as they reach the pack house (Mencarelli & Bellincontro 2005). Depending on market request, grape bunches are packed within a day. Thereafter, grapes are placed in cold storage at recommended temperatures of between 0°C and -2°C (Mencarelli & Bellincontro 2005). Cold treatment schedules may be used to control specific pests associated with shipments of fruit to export markets (USDA Treatment Manual 2014). For temperature treatments of harvested table grapes specifically, treatments are limited to cold storage (Sharp 1993).

Temperature treatments are, for the most part, environmentally safe and no toxic or chemical residues are generated (Sharp 1993). Cold and heat treatments are used to eliminate undesirable pests of quarantine significance, without affecting the quality or condition of the

host commodity, an aspect important to consider when supplying any fruit to foreign markets. The precise determination and application of temperature and the duration of exposure are considered to be the most critical factors associated with postharvest temperature treatments as narrow margins exist between the low and high temperatures needed to exterminate the pest and the tolerance of the commodity to the applied treatment. Other postharvest temperature treatments, applied for the control of pests and plant pathogens, include exposure to cold (0°C – 2.22°C), water or air (> 45°C), dry heat (> 60°C), vapour heat and quick freezing (< -17°C) (Sharp 1993).

A cold treatment consists of two steps; precooling the fruit to its very centre to a specified temperature (prior to export), and holding the fruit at that temperature for a specified period of time (Richardson 1952). According to PPECB Protocols (PPECB-HP27 2013), grapes are best stored at a pulp temperature of -0.5°C with a relative humidity of 90% to 95% in order to minimise decay and desiccation of stems and berries. It is stated that pre-cooling should be applied within six hours after harvest to avoid incidents of decay, dry/brown stems, soft berries and moisture loss. Consignments intended for export (via marine shipment) are typically packed in polyethylene bags which are placed in packing cartons. A slow-release sulphur dioxide sheet is placed on top of these berries within a carton to deter the establishment of mould. For both conventional vessels and all containers, grapes in transit must not reach a pulp temperature higher than 2.5°C.

When export markets are considered cold-sterilization is, in most cases, mandatory. It is an inexpensive mitigation treatment typically used to control *Ceratitis capitata* (Wiedemann) (Mediterranean fruit fly) and *Thaumatotibia leucotreta* (Meyrick) (False Codling Moth) (Witbooi & Taylor 2008). The current cold-sterilization period for table grapes for the control of these pests (among others) is 22 days at -0.55°C (T107-e) (USDA Treatment Manual 2014).

The aim of this chapter was to assess the efficacy of PPECB cold-sterilization protocols used for the control of False Codling Moth and *Ceratitis* species (i.e. Mediterranean fruit fly, *C. quinaria* Bezzi, *C. rosa* Karsch) for the control of *Holocacista capensis*. In addition, pesticide use and biological control agents, used for the control of other leafmining pests, will be considered and discussed.

4.2. Materials and methods

4.2.1. Study sites

This study was conducted in the Drakenstein region of the Cape Winelands at Optenhorst (33°43'03.8"S 18°57'55.8"E), a table grape farm in the district of Paarl in the Western Cape Province of South Africa.

4.2.2. General field observations

Emerged parasitoids (from larval and pupal samples collected in the field, see Chapter 2) and the influence of spray programme (Appendix 2) were monitored and analysed throughout the grapevine growing seasons. Red Globe bunches covered by berry bunch caps, used for the protection of berries from avian predation (physical control measure), at St Malo, were monitored (for rooted cocoons) on an ad hoc basis.

4.2.3. Preliminary temperature treatments

4.2.3.1. Cold store experiments

Six hundred and eighty newly descended cocoons, collected from Optenhorst in March 2015, were placed individually in aerated 1.5 ml microfuge tubes. Twenty four hours after collection (kept at room temperature), the microfuge tubes were placed in five boxes of grapes (each containing 9.5 kg of grapes) stored in a cold room set at a cooling temperature of -0.5°C (an exact temperature of -0.55°C could not be attained due to the particular cold storage facilities). One hundred and nineteen microfuge tubes were placed in each box. Treatments included the removal of 17 cocoons from cold storage at seven time periods (i.e. after 11, 18, 20, 22, 24, 26 and 33 days of cold temperature exposure). Cocoons removed from the cold room were kept at room temperature for 24 hours before being placed in a temperature controlled incubator (MRC LE-509, Holon, Israel) set at 25°C. Eighty five of the 680 cocoons were stored in five separate bags (17 microfuge tubes per bag) and placed in a 25°C rearing chamber (incubator) after being kept at room temperature for 24 hours after collection (control). Microfuge tubes were monitored regularly and eclosed cocoons were counted.

4.2.3.2. Water bath experiments

Ninety nine newly descended larval cocoons, collected from Optenhorst in May 2015, were placed individually in aerated 1.5 ml microfuge tubes. Due to unforeseen circumstances, cocoons could not be placed in a water bath 24 hours after collection and had to be stored in a 25°C rearing chamber whilst a water bath was prepared. Thirteen days after incubation, cocoons were placed in an 11°C rearing chamber to avoid eclosion. Seventeen days later, cocoons were removed from the chamber and stored at room temperature in the laboratory for 24 hours before being placed in a programmable, circulating water bath (Huber cc410-wl, Offenburg), set to -0.5°C for 22 days (according to PPECB protocols for the cold sterilization treatment of FCM and *Ceratitis* species). Twenty two days later, cocoons were kept at room temperature for 24 hours before being placed in a 25°C rearing chamber.

Another 99 cocoons were collected for a control treatment, and treated in a similar fashion as the control in the previous section (4.2.3). Cocoons were monitored regularly and total mortality recorded.

4.3. Results

4.3.1. General field observations

In the 2013/2014 and 2014/2015 grapevine growing seasons, farmers reported decreases in leafminer abundance after the application of dichlorvos in December and January in 2013 and 2014, respectively (Chapter 2, Appendix 2). Of the three farms monitored, St Malo yielded the highest abundance of leafminers in the 2013/2014 grapevine growing season. In October 2014, preventative applications of spinetoram were applied to heavily infested and surrounding blocks at St Malo. Four applications were administered from October to December 2014 on Red Globe, whilst only three were applied from October to November 2014 on Regal Seedless. Infestation was considerably lower in the 2014/2015 grapevine growing season than in the 2013/2014 grapevine growing season. An average of ca. 361 moths were recorded per sticky trap on a fortnightly basis throughout the 2014/2015 grapevine growing season (September 2014 – May 2015) at St Malo whereas an average of

ca. 1381 and ca. 803 moths were caught per sticky trap on a fortnightly basis at Optenhorst and Monte Cristo, respectively.

A variety of parasitoid wasps associated with *H. capensis* were identified from larval and pupal samples gathered in the field (Chapter 2). These parasitoids included two species of eupelmids, an eulophid species, a mymarid species and two eurytomid species. Based on the relative abundances of the parasitoids emerging from both larval mines and pupal cocoons, it is unclear if parasitoids show any preference to a particular life stage. In the case of the eulophid and mymarid species, however, many of the samples were collected from cocoons and mines, respectively.

Throughout the 2013/2014 growing season, individual berry bunches, in a small area located at the edge of one of the Red Globe blocks, were covered by dense mesh to deter feeding birds. Throughout the season, these bunches were monitored regularly. No cocoons were present on these bunches throughout the grapevine growing season.

4.3.2. Laboratory experiments

Cocoons reared at 25°C (control treatment) experienced the lowest levels of mortality (ca. 15% of collected samples) (Fig. 4.1). One hundred percent mortality of pupae occurred at 26 and 33 days of exposure to -0.5°C. At 11 days of exposure, ca. 55% of cocoons survived to adulthood. Exposure ranging from 18 to 24 days yielded ca. 85% - 96% mortality.

The largest number of parasitoid wasps were collected from the control treatment where approximately 12% of the cocoons were parasitized (Fig. 4.2). Only one percent of individuals yielded parasitoids after 11 to 22 days of exposure to -0.5°C. No parasitoid wasps were detected after 22 days of exposure to -0.5°C (i.e. 24, 26 and 33 days of exposure to cold treatment).

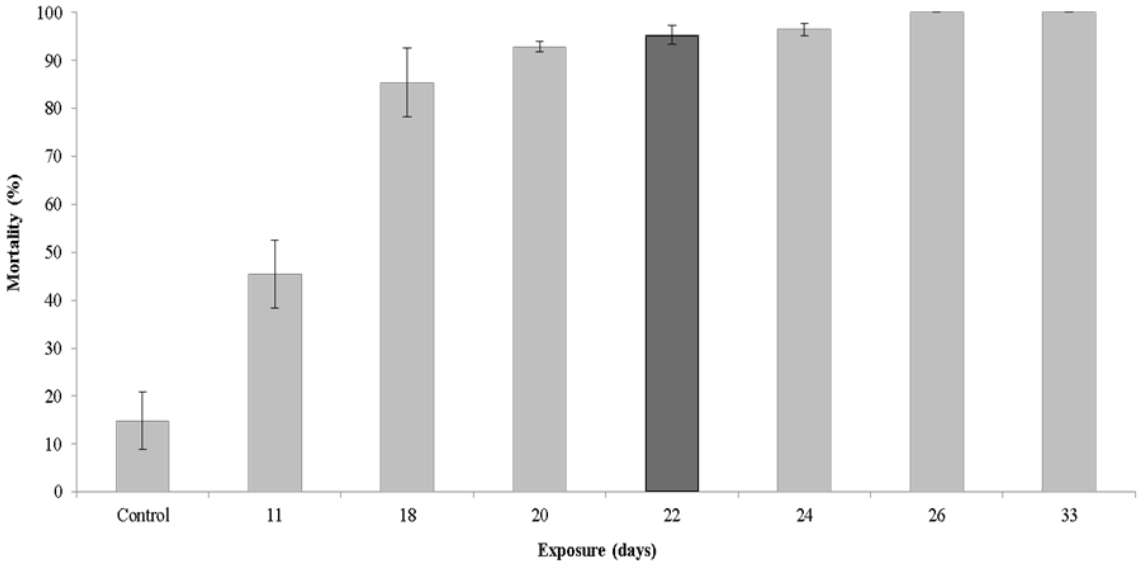

Figure 4.1: The average mortality of *Holocacista capensis* cocoons (± S.E.) recorded after exposure to -0.5°C for various durations (11 – 33 days exposure) (standard treatment highlighted). Control cocoons were exposed to 25°C rearing temperatures throughout the study.

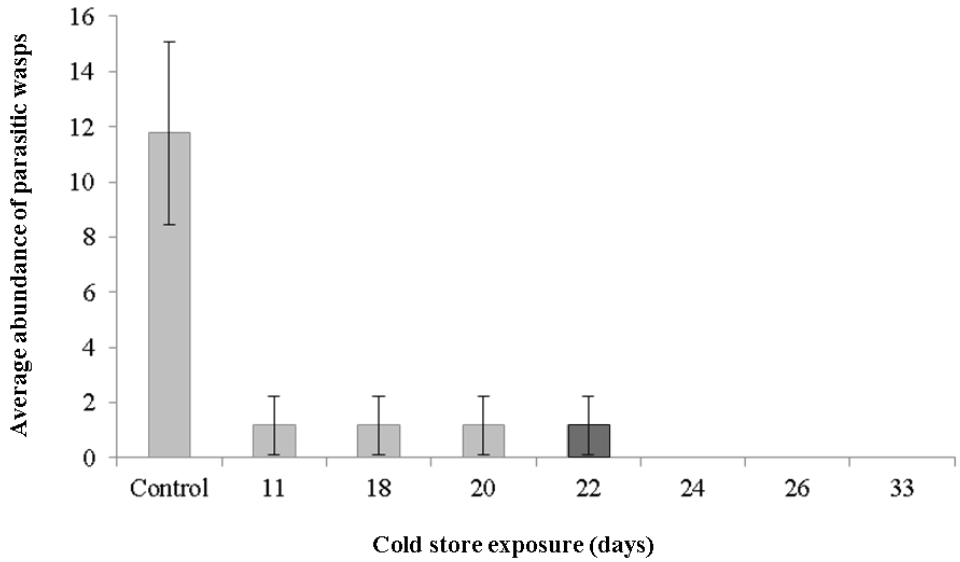

Figure 4.2: The average abundance of eclosed parasitoid wasps (± S.E.) after exposure to -0.5°C for various durations (11 – 33 days exposure) (standard treatment highlighted). Control cocoons (*Holocacista capensis*) were exposed to 25°C rearing temperatures throughout the study.

Of the cocoons exposed to the water bath cold treatment, 91.92% of cocoons eclosed. Of the control individuals, 97.98% of cocoons eclosed. No parasitoid wasps from these samples were detected.

4.4. Discussion

A study by Yokoyama *et al*. (2001) recorded 100% mortality of *Frankliniella occidentalis* Pergande (Western flower thrips), *Tetranychus urticae* Koch (two-spotted spider mite) and *Platynota stultana* Walshingham (Omnivorous leafroller) in packed table grape cartons, exposed to a combination of slow release sulphur dioxide pads and low temperature storage for eight weeks. The cold store experiments, conducted in this study, strongly suggest that a cold-sterilization treatment of -0.5°C for 22 days will not be sufficient for the control of *H. capensis* as 100% mortality occurred only on the 26th day of continuous exposure to -0.5°C. It is suggested that cold-sterilization treatments could be considered if exposure exceeds or equals 26 days of exposure to -0.5°C. The temperatures in the cold store did, however, fluctuate depending on the activity in and around the cold storage unit, and temperatures often deviated around the set point by at least ±0.5°C. However, a study conducted by Powell (2003) on *C. capitata*, suggested that the duration of cold treatment was more important than the storage temperature.

Before further studies are considered, a trial should be conducted under better controlled cold storage conditions (set at -0.55°C and -0.6°C with little variation similar to consignment treatments intended for export). If similar results are obtained, further studies aimed at determining probit 9 quarantine security should be conducted. To be considered as a viable means of control, potential quarantine treatments must provide probit 9 quarantine security (i.e. 99.9968% mortality) (Sharp 1993).

In the case of the water bath experiments where cocoons underwent pre-treatment cooling temperatures, very high incidents of survival were recorded. This finding suggests that *H. capensis* has the ability to develop cold hardiness, although freeze-tolerance cannot be inferred (Bale 1993). Future studies should be conducted to determine their supercooling point and lower lethal thermal limit in order to establish their low temperature tolerance and cold hardiness (see Klok *et al*. 1997; Powell 2003; Boardman *et al*. 2012). In the event that cold hardiness develops in *H. capensis*, concern should be raised in the few, exceptional cases where grapes from the Orange River and Vredendal vine growing regions are pre-cooled to 5°C or colder before being transported in refrigerated trucks for shipment (PPECB-HP27 2013). Despite the fact that cold storage facilities are now readily available in these areas, the deviation in temperature protocols was established in the 1990s when there were

few cold storage facilities available to farmers in the Orange River and Vredendal regions. This protocol still stands when high densities of harvested grapes cannot be housed in appropriate cold storage facilities due to space limitations (B. Henning *pers. comm.*).

In most commercial orchards insecticides are the main defence against leafminers and other insects (Maier 2001). Many researchers have explored the efficacy of a variety of chemical products used for the control of economically important leafmining pests in field and laboratory conditions (see Beattie *et al*. 1995; Rae *et al*. 1996a; Rae *et al*. 1996b; Villanueva-Jiménez *et al*. 2000; Lietti *et al*. 2005; Zhao *et al*. 2002; Zhao *et al*. 2006; Elbert *et al*. 2008). Currently, no chemical products have been registered or can be recommended for the control of *H. capensis*. The findings of this study, however, suggest that there may be preventative chemical control measures that can be taken to avoid *H. capensis* infestation. The use of the organophosphate, dichlorvos, should be avoided altogether due to the deleterious effects to both humans (and other fauna) (as an acetylcholinesterase inhibitor) and the environment (by accumulation of environmental pollution) (see Chuiko 2000; Eddleston *et al*. 2008) (Colovic *et al*. 2013).

Spinosyns (derived from fermented bacteria) have been registered on over 180 crops in over 35 countries for the control of a variety of Lepidoptera (including leafminers), Coleoptera and Thysanoptera (Zhao *et al*. 2002). In the case of *H. capensis*-infested farms, spinosyns could be applied as a preventative application or, alternatively, as a curative application early in the season (J. de Waal *pers. comm.*). This statement is further supported by the considerable decrease in leafminer abundance on St Malo after the preventative application of spinetoram in the early months of the 2014/2015 grapevine growing season. Area-wide application of spinetoram would not, however, eradicate *H. capensis* from South Africa as native and untreated populations are likely to infest or re-infest cultivated areas. Chemical applications such as these would, therefore, need to be applied regularly to effectively control leafminer infestation. Further research in this regard is required.

Many eulophids parasitize leafmining Lepidoptera and Diptera (see Hills & Taylor 1951; Bigger 1973; Peña *et al*. 1996; Rauf *et al*. 2000; Chen *et al*. 2003; Mujica & Kroschel 2011). The parasitic leafminer generalist *Chrysocharis gemma* (Walker) (Hymenoptera: Eulophidae) causes high larval mortalities of *Phytomyza ilicis* Curtis (Diptera: Agromyzidae) and is absent in the colder regions of the European distribution range (Klok *et al*. 2003). Results obtained

by Sugimoto *et al*. (1987) and Sugimoto & Tsjimoto (1988) regarding the use of marking pheromones by parasitoid wasps suggests that parasitoid wasps employ an area-concentrated search, as discussed by Laing (1937), in which search efforts are affected primarily by ideal environmental conditions and secondarily on the identification of hosts (Sugimoto *et al*. 1990). Pilot trials have indicated that there are several parasitoids of *H. capensis* which attack both the larval and pupal life stages of the leafminer.

Due to the fact that mymarids parasitize many species of Hemiptera, Lepidoptera, Coleoptera, Diptera and Psocoptera, and as mymarids are all primary endoparasites of insect eggs (Hills & Taylor 1951; Prinsloo 1980), studies should be focused on the ecology of the other hymenopteran parasites collected from larval mines and pupal cocoons in this study (namely eupelmids, eulophids and eurytomids) and the potential use of each parasite as a biological control agent. In some cases, parasites do not have the ability to effectively control leafminer pest populations. A study conducted by Heads & Lawton (1983) found that pupal parasites failed to effectively control *Phytomyza ilicis*, and thus failed as potential biological control agents. As a result, all parasites associated with *H. capensis* should be screened and tested for their efficacy at reducing leafminer population abundance.

If leafminer infestation cannot be avoided throughout a grapevine growing season, it may be necessary to design a cap or covering that may be fitted over a bunch of grapes to avoid the establishment of cocoons on berries (H. Geertsema *pers. comm*.). Alternative cultural methods (based on infestation incidents and cultural methods observed in marginally infested farms/blocks) for heavily infested blocks include the removal of bark from grapevine stems in the winter season to remove as many overwintering cocoons as possible coupled with the placement of pheromone traps in the early season to reduce adult male population abundance.

Due to the large numbers of *H. capensis* occurring in table grape vineyards, in severely infested farms, it is likely that pre-harvest control measures may not suffice to maintain international export markets. For this reason, various post-harvest treatments should be investigated. These strategies/treatments should, ideally, also be tested in conjunction with fruit quality experiments.

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Chapter 5

General discussion

A high abundance of *Holocacista capensis* van Nieukerken & Geertsema (2015) (the Cape grapevine leafminer), a pest in table grape vineyards, was reported as recently as 2012 in areas surrounding Paarl, Western Cape. To date, infestations have been noted when leafminer infestation has progressed to the contamination of berry bunches, which has led to the use of a variety of costly and environmentally-damaging chemical applications which seem to have little effect on leafminer populations as they are typically applied in the late stages of a grapevine growing season. When the larval leafmining stage of the life cycle is complete, the larvae descend from the leaves in cocoon casings constructed from the epidermal layers of the infested leaves from silken threads, and silken mats will be produced to keep the cocoons firmly attached to any surface encountered (e.g. a trellis post, a bunch of grapes, another leaf etc.). In cases where *H. capensis* is considered a phytosanitary pest, management action associated with chemical applications is superfluous after any bunch infestation has occurred unless labour intensive, post-harvest management strategies are carried out.

The aim of this study was to gain insights into the biology of *H. capensis* to facilitate potential monitoring and management strategies. When investigating the biology of *H. capensis* several aspects were studied. These aspects included: the investigation of the seasonal cycle and seasonal occurrence of adult moths (i.e. male moths) and leafmining larvae, respectively; the identification and brief investigation into the parasites associated with the larval and adult stages; the investigation of environmental, structural and spatial aspects that may affect leafminer prevalence as well as the investigation of the distribution of *H. capensis* throughout the Western Cape, South Africa. Monitoring strategies based on the generic pest monitoring system described by de Villiers and Pringle (2007) were explored and several management strategies were discussed.

Temperature is one of the most influential factors that affect the developmental rate of all arthropods (Briere *et al*. 1999). Due to observed behaviours of feeding larvae and correlations noted between the emergence of adult moths and average temperatures, it can be assumed that temperature plays a vital role in the development of pupae, and the resultant number of generations produced within a season. Larval and pupal life stages of *H. capensis*

experienced faster development under warmer conditions, whilst under colder conditions decreased developmental rate was experienced or diapause was induced in both life stages. Pilot trials did, however, indicate that average temperatures exceeding 30°C led to increased pupal mortalities. Based on the conservative estimate of a seven week life cycle, a minimum of four generations can be present within a grapevine growing season if the average season is assumed to last approximately 26 weeks (i.e. mid-October to the end of April).

Using sticky pads coupled with pheromone dispensers, synthesized with the female-produced sex pheromone identified by Wang *et al*. (2015), adult male moths can be detected throughout the year in infested stands of *Vitis vinifera*. The implications of the presence of adult male moths throughout the colder winter months are varied. Firstly, it is important to note that these adults may inhabit alternative plant hosts such as ground cover or adjacent stands of vegetation, although no literature has explored alternative-host overwintering habits of leafmining insects. In most cases, leafminers overwinter as pupae (Chen & Kang 2004; Chen & Kang 2005), although some leafmining species have been known to overwinter in the adult form (Wagner *et al*. 2012). *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) has the ability to overwinter in the egg, pupal or adult stage, although it is believed that the larvae feed almost continuously and do not enter diapause, whilst developmental and reproductive diapause has not yet been investigated in detail (Van Damme *et al*. 2015). It is highly likely, however, that adults eclosed under cooler conditions undergo diapause and contribute to the first generation of leafminers in the next grapevine growing season when conditions become favourable to do so.

Male moth trap counts indicate a continuous presence of moths in the early months of a grapevine growing season which is likely to be as a result of fine differences in microclimatic conditions which may affect the eclosion rate of overwintering pupae. In the warmer summer months, this phenomenon could cause observed multivoltine behaviours. Princebourde *et al*. (2007) noted that the developmental rate of larvae of the leafmining moth *Phyllonorycter blancardella* (Fabricius 1781) (Lepidoptera: Gracillariidae) occurring within the canopy of apple trees, were significantly affected by varying microclimates caused by shaded and sunlit conditions. Due to the fact that adult moths are present before budbreak, leafmining larvae are inevitably found soon after vine leaves are present. As a result of the former, the first generation of larvae will be produced over a period of a few weeks.

A study conducted by Faeth *et al*. (1981), on a variety of leafmining insects occurring on oak species, found that densities of active mines varied significantly both seasonally and between years. This phenomenon will be greatly affected by temperature and potentially by grapevine quality (determined by water availability, the level of parasitism, soil quality etc.) which has direct implications on the voltinism of a leafminer (Faeth *et al*. 1981; Faeth 1985). Similar trends were noted in this study. Adult moths and larval leafminer abundance varied greatly between blocks and between growing seasons which suggests that early season monitoring efforts are essential to detect potential leafminer infestations within a growing season.

The present study indicated potential differences between edge and internally located plots as well as between different types of trellis systems. In cases where differences are observed, environmental factors such as microclimate, shade and soil quality may play an important role in such phenomena. Incongruencies such as these and trends in the seasonal abundance of the pest play an important role when monitoring systems are considered.

The distribution of *H. capensis* extends from Oudtshoorn to Vredendal in the Western Cape and is generally west-skewed. The distributional ranges of other leafminer species may be closely correlated with those of *H. capensis* and the distribution of the pest and its relatives should, therefore, be explored throughout southern Africa. The host-plant switch from the native Vitaceae to cultivated stands of *Vitis vinifera* may very well have taken place as a result of habitat loss and long-term deviations in climatic conditions (i.e. altered species interactions) (see Menéndez 2007; Netherer & Schopf 2010). The relationship between leafminer abundance and temperature fluctuations should be further explored to predict further range changes, and the potential threat of establishment and proliferation at export destinations.

It is likely that populations established on native and ornamental varieties of Vitaceae in nurseries and home-gardens (including ornamental vines growing in communal areas for aesthetics, e.g. fuel stations and public parks) contribute to infestations experienced on surrounding table grape producing farms. Once viable control measures have been established, control of these areas by the application of control measures (such as those discussed in this thesis) should be considered for the area-wide management of *Holocacista capensis*.

Based on observations made throughout the last three grapevine growing seasons and knowledge accumulated thus far, preventative chemical control efforts focused on preventative strategies in the early season have been the most successful control efforts. An effective monitoring system would, however, reduce the need to administer preventative applications. In doing so, resistance to preventative chemical applications may be avoided. Due to the high variability of leafminer occurrence between season, farms and cultivars, however, it is recommended that pre-emptive measures be taken to avoid progressive infestation in cases where *H. capensis* is considered a phytosanitary risk.

It is important to consider that leafminer abundance increases as the season progresses (see Chapter 2), and that growers have, in the past, struggled to control leafminer infestation once noticeable leaf and bunch infestation has been detected, despite late-season chemical applications (see Chapter 4). Early-season pheromone trapping could be used to effectively monitor leafminer adult populations and reduce the need for growers to delegate leaf inspection tasks to farm labourers when many infested blocks are to be monitored. The findings of the present study suggest that less than 85 moths caught per block per fortnight should warrant preventative management action when 1% bunch infestation is tolerated. If pheromone dispensers could be supplied to growers before the end of a growing season, growers may be able to predict leafminer infestation in the proceeding grapevine growing season (i.e. if adult male moths are in high abundance at the end of a growing season, it is highly likely that severe infestation will be experienced in the following season, especially in the absence of post-harvest management strategies). Preventative measures associated with adult abundance would work most effectively when applied close to budbreak to avoid the establishment of leafmining larvae. Parasitism of larvae and cocoons was relatively low, based on field collected data (<12%). This would, therefore, not exert enough control for management purposes. Supplementary releases would, therefore, need to be considered. If any leafminers are detected, the release of species-specific parasitoid wasps in the early season may also improve wasp abundance later in the season. This would need to be coupled with a reduction in pesticide usage, which is mostly likely the reason for the low natural parasitism observed.

A study by Trumble (1985) found that weekly applications of avermectin, cyromazine and methomyl elicited varied responses from both *Lyriomyza trifolii* (Burgess) (Diptera: Agromyzidae) and several associated parasitic species on *Apium graveolens* L. (celery). Avermectin and cyromazine effectively suppressed leafminer populations, however, the use

of cyromazine significantly reduced the survival rate and emergence of immature parasites [similar trends associated with the application of abamectin on celery infested by *L. huidobrensis* (Blanchard) (Weintraub & Horowitz 1998)]. Methomyl increased the abundance of leafmining larvae and decreased the survival of adult parasites. The use of cyromazine and methomyl are, therefore, not conducive to simultaneously reducing leafminer populations and establishing an appropriate management strategy that involves the use of parasites in an Integrated Pest Management (IPM) orientated strategy (Trumble 1985). Several publications have emphasised the need for appropriate IPM strategies to reduce the reliance on chemical management which, in some cases, has led to pesticide resistance and the suppression of natural enemies (see Shanower *et al*. 1993; Maier 2001; Zhao *et al*. 2002; Lietti *et al*. 2005; Zhao *et al*. 2006).

Before management strategies are considered, the short-falls of conventional pest management strategies dominated by the application of chemical treatments need to be considered and addressed (National Research Council 1996). Civelek & Yoldaş (2003) question the use of insecticides for the control of *L. huidobrensis* and *L. trifolii* in cucumber greenhouses in the Menderes region of Turkey as overall differences in leafminer populations between insecticide-treated and non-treated flora were minimal, whereas parasite populations were significantly reduced in cases where insecticides (i.e. chlorfenapyr, thiocyclam hydrogen oxalate, methadimophos and imidaclorpid) were used. It was suggested that alternative methods should be developed to increase and protect parasitoid populations which could effectively control leafminer populations whilst being economical and avoiding an influx of chemicals into the environment.

Strategies such as these are considered to be short-term solutions which are, in many cases, harmful to both humans and the environment. In the case of *H. capensis*, where seasonal infestation on commercial vines is most likely replenished continuously by local source populations, overwintering pupae and/or commercially bought scions, it is highly likely that complete eradication of the pest in vineyards is unattainable and the continuous application of harsh chemicals may lead to the reduction of natural enemies and resistance against pesticides in leafminers and other pests (Shanower *et al*. 1993; Maier 2001; Zhao *et al*. 2002; Zhao *et al*. 2006; Lietti *et al*. 2005). Growers should, therefore, aim to manage leafminer infestation using sustainable and appropriate long-term solutions. Adopting chemical deficient management strategies will, in addition, eliminate incidents of resistance in pest populations such as those seen in studies by Macdonald (1991) and Fragoso *et al*. (2002).

Azadirachtin, a neem kernel extract belonging to the tetranortriterpenoid organic molecule class, exhibits potent insect antifeedant and insect growth regulator effects on the immature stages of insects (Mordue & Blackwell 1993; Immaraju 1998) and can effectively control leafmining insects (Weintraub & Horowitz 1997; Immaraju 1998). As a reduced-risk biopesticide, azadirachtin is an ideal addition to an Integrated Pest Management (IPM) strategy (Immaraju 1998) and should be further studied as a potential control mechanism for *H. capensis*.

Mating disruption may also be considered for the control of *H. capensis* and further studies should be aimed at determining the efficacy thereof. Mating disruption for populations of *Tuta absoluta* Povolny (Gelechiidae) and *Phyllocnistis citrella* Stainton (Gracillariidae), two leafmining moths, have been studied in greenhouse conditions, although further research is required to determine the efficacy of mating disruption for leafminers in outdoor field conditions (Mafi *et al*. 2005; Vacas *et al*. 2011). Studies by Head *et al*. (2000) and Batalla-Carrera *et al*. (2010) have proven that entomopathogenic nematodes have the ability to successfully control leafminer populations and thus should also be considered for the control of *H. capensis*.

Elsewhere, ripening grape bunches are susceptible to hail and bird damage. To counter damage by these agents, vines are either covered by shade cloth or developing grape bunches are hooded with a shield or cap. When the feeding stage of the larvae of *H. capensis* has ended, the larva excises a double cocoon shield for eventual pupation and descends after excision of the cocoon from the leaf from the canopy to pupate on any obstacle, be it a bunch, berry, trellis or even another leaf. The use of shields as a protective measure to eliminate cocoon contamination by "catching" descending larvae, has been suggested and has met with favour and considered practical by some table grape producers in the Western Cape (H. Geertsema *pers. comm*.), although to date this protective measure has not been applied in this region. The cost and application of these shields (which are available in the trade) will offset the labour costs and bunch rejections incurred in the packing shed. This particular control method is not only environment-friendly, but will also eliminate the suggested cold-treatment of contaminated bunches during export. If cold-storage is designed to kill the cocoons on export grapes, the problem remains of the presence of dead cocoons on the berries itself. The use of shields during the developing and ripening stage of the grapes appears to be the only viable option of control in the absence of chemical applications, as clean berries are desired by the ultimate consumer.

Irradiation involves the exposure of a commodity to a controlled amount of ionizing energy for a specified time period, to sterilise or disinfest insects of quarantine importance (Witbooi & Taylor 2008). Doses of 250-600 Gy have been found to control Tortricidae, Pseudococcidae, Curculionidae and Tephritidae (either by killing or by sterilization) without adversely affecting the quality of grapes, pears and apples (Pryke & Pringle 2008). As a result, irradiation as a means of postharvest disinfestation has great potential (for deciduous fruits specifically). Further investigation into the irradiation of grapes, infested by *H. capensis* cocoons, should be carried out. However, the problem of cocoons, alive or dead, as a contaminant to berries, remains a problem.

Controlled atmospheres are commonly used to extend the storage life of deciduous fruit (Pryke & Pringle 2008). There are very few studies that have explored the effects of various atmospheres on fruit and insects, however, it appears that higher temperatures in controlled atmospheric treatments are more successful in controlling insect pests than at lower temperatures (Pryke & Pringle 2008).

Bioassays performed by Leroy *et al*. (2000) investigated the effect of genetically modified coffee plants (*Coffea canephora* and *C. arabica*) on *Perileucoptera coffeella* and other *Leucoptera* species (Lepidoptera: Lyonetiidae). It was found that transformed plants, using a synthetic version of the *cry*1Ac gene of *Bacillus thuringiensis*, conferred resistance to leafminers. Globally, the pest status of many leafminers associated with *Vitis vinifera* have become realised over the last few decades, control measures (such as those mentioned above) should be explored and may be beneficial to all regions affected by leafminer infestation on cultivated stands of *V. vinifera*.

The findings of this research can be used to facilitate monitoring and management strategies associated with the control of *H. capensis* in accordance with appropriate IPM strategies. Growers can now make informed decisions on structural and physical elements (i.e. trellis systems and utilizing caps over bunches), to prevent leafminer infestation, and on monitoring data pertaining to action thresholds using pheromone traps.

Future studies should be focused on the regional distribution of *H. capensis* throughout southern Africa in cultivated stands of *V. vinifera* as well as in natural stands of Vitaceae to gain a greater understanding of their invasion potential in surrounding areas. Additional studies should be conducted to determine the ecology of *H. capensis* on native Vitaceae and

the driving factors that influence the host plant shift. The correct identification of *H. capensis* in studies such as these should be conducted by the use of DNA barcoding. Studies aimed at determining the attractiveness of the pheromone to other native heliozelids should also be considered.

Management associated with alternative control methods, such as biological control agents (i.e. parasitoid wasps), needs to be further investigated, by assessing optimal environmental conditions needed for improving biological control; as well as surveying for other natural biological control agents, such as entomopathogenic- nematodes and -fungi. Post-harvest control should be focussed on assessing temperature treatments coupled with modified atmosphere conditions (low oxygen/high nitrogen) as well as heat treatments (Neven & Mitcham 1996) and using fumigants such as ethyl formate (Simpson *et al*. 2007).

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Appendix 1

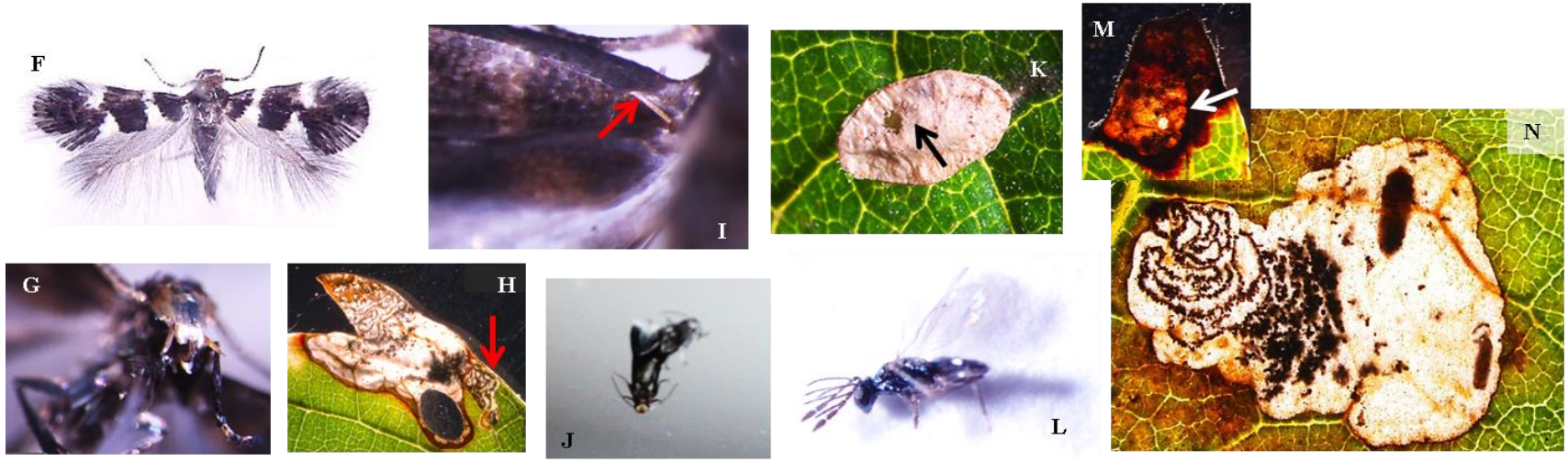
Table 1.a: A visual guide to the life cycle and morphology of *Holocacista capensis* and the typical damage symptoms of infested grapevines. Damage symptoms: on leaves, berry bunches and trellis posts (A), larval mines on grapevine leaves and clustered mines on Ramsey rootstock (B) as well as the presence of cocoons on vine stems, the formation and establishment of cocoons and eclosed cocoons (C).

A. DAMAGE SYMPTOMS

B. LARVAE

C. COCOONS

Table 1.a (cont.): The differences in the wing pattern (antemedial line of forewing) (D) and abdomen (E) of male and female moths, a pinned female moth (F), the morphology of the head (G), the sometimes clustered larval habit (H), the frenulum wing coupling (I), mating behaviour (J), a parasitized cocoon (K), a parasitic eupelmid (collected from a cocoon) (L), a parasitized larval mine (M) and a parasitized larval mine (dead larvae and pupae of a parasitoid wasp present) (N).

<table>
<tr><th colspan="4">ADULTS</th></tr>
<tr><th colspan="2">D. WING PATTERN</th><th colspan="2">E. ABDOMEN</th></tr>
<tr><th>MALE</th><th>FEMALE</th><th>MALE</th><th>FEMALE</th></tr>
<tr><td colspan="2"> 
</td><td colspan="2">  </td></tr>
<tr><th colspan="4">GENERAL MORPHOLOGY, BEHAVIOURAL ASPECTS AND PARASITISM</th></tr>
<tr><td colspan="4"> 
</td></tr>
</table>

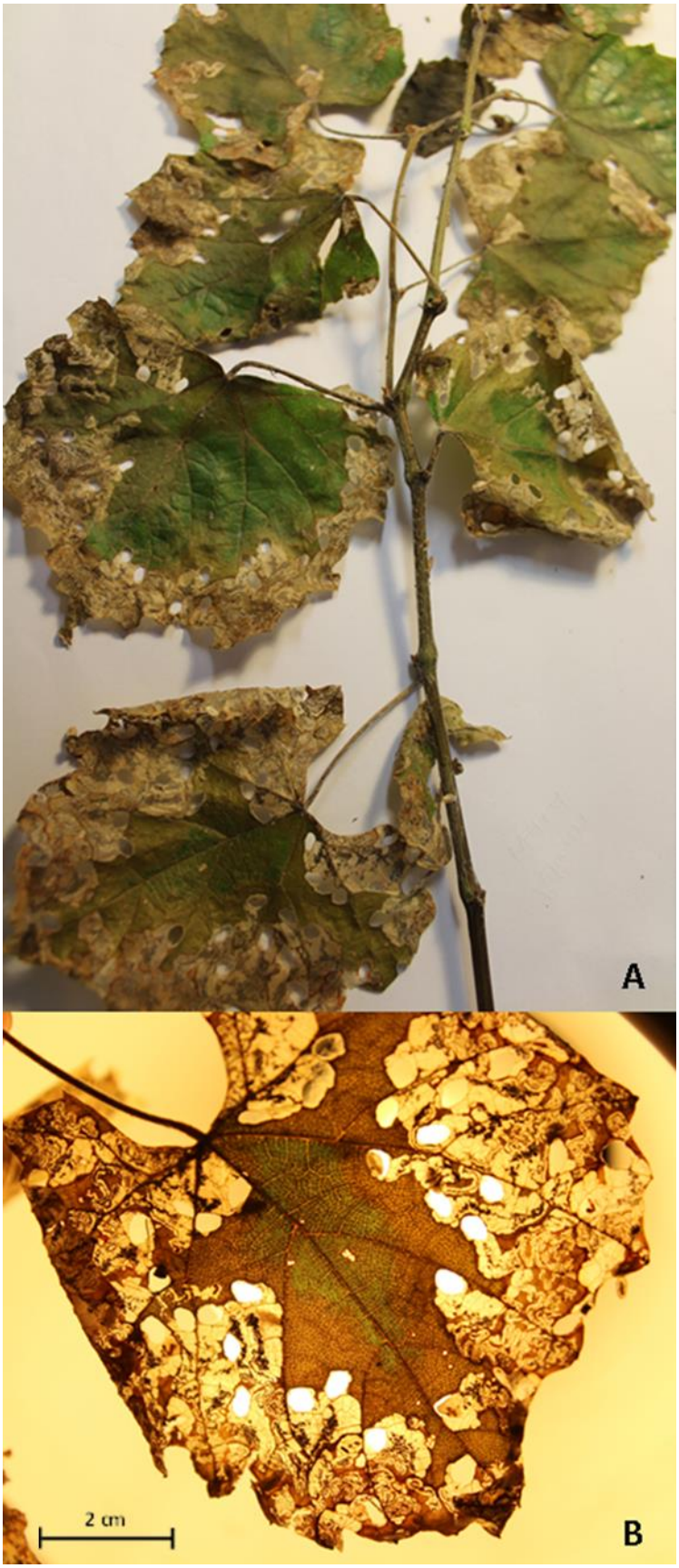


Figure 1.a: A shoot (A) originating from Ramsey rootstock retrieved from Optenhorst (Dan Ben Hannah) in 2015. The infestation can be better observed when illuminated from underneath the leaf (B).

Appendix 2

Table 2.a: Cultivars, plant dates, block sizes and soil types of blocks sampled fortnightly in St Malo, Monte Cristo and Optenhorst.

FARM AND BLOCK DETAILS					
FARM	**CULTIVAR/CODE**	**YEAR PLANTED**	**SIZE OF BLOCK (HA)**	**SOIL-TYPE**	**NOTES**
Monte Cristo	Crimson (RG)	2002	3.97	Clay-loam	Removed between 2014 and 2015 due to bacterial blight (*Xylophilus ampelinus*) infection, currently fallow, studied as an ad hoc site in 2014 only.
	Regal (HR)	1998	0.78	Clay-loam	
	Crimson (RRG)	2006	1.83	Clay-loam	
Optenhorst	Dan Ben Hannah (DBH)	1985	1.8	Sand-loam	
	Alphonse (AL)	1985	0.9	Sand-loam	
	Crimson (CR)	2006	0.9	Sand-loam	
St Malo	Red Globe (AGR)	2000	0.55	Sand-loam	Removed in 2015 (March) due to bacterial blight (*Xylophilus ampelinus*) infection, to be replaced with Sugra 35.
	Red Globe (GR)	1994	1.01	Sand-loam	Studied as an ad hoc site in 2014 only, block removed in October 2015
	Regal (R)	1999	1.1	Sand-loam	
	Regal (AR)	1999	1.1	Sand-loam	

Table 2.b: Spray programme of blocks/cultivars sampled fortnightly in St Malo, Monte Cristo and Optenhorst.

SPRAY PROGRAMME					
FARM	**SEASON**	**CULTIVAR**	**DATE**	**ACTIVE CHEMICAL**	**TARGET**
Monte Cristo	2013/2014	Crimson Seedless	23-Oct-13	Spirotetramat	Pseudococcidae
			05-Dec-13	Spirotetramat	Pseudococcidae
		Regal Seedless	09-Jan-14	Dichlorvos	Pseudococcidae
	2014/2015	Crimson Seedless (35)	05-Nov-14	Spirotetramat	Pseudococcidae
			06-Nov-14	Lambda-cyhalothrin	Curculionidae
			03-Dec-14	Spirotetramat	Pseudococcidae
			09-Jan-15	Dichlorvos	Pseudococcidae
		Crimson Seedless (37)	17-Nov-14	Lambda-cyhalothrin	Curculionidae
			24-Dec-14	Dichlorvos	Pseudococcidae
Optenhorst	2013/2014	Alphonse Lavallée	4-Oct-13	Sulphur	Not disclosed
				Fenazaquin	
			11-Oct-13	Spirotetramat	
			23-Oct-13	Fenazaquin	
			3-Dec-13	Spirotetramat	
			10-Jan-14	Dichlorvos	
		Crimson Seedless	10-Oct-13	Sulphur	
			18-Oct-13	Sulphur	
				Fenazaquin	
			24-Oct-13	Spirotetramat	
			3-Dec-13	Spirotetramat	
			20-Jan-14	Dichlorvos	
		Dan Ben Hannah	10-Oct-13	Sulphur	
			17-Oct-13	Sulphur	
				Fenazaquin	
			23-Oct-13	Spirotetramat	
			2-Dec-13	Spirotetramat	
	2014/2015	Alphonse Lavallée	15-Sep-14	Sulphur	
				Methomyl	
			22-Sep-14	Sulphur	
				Methomyl	
			01-Oct-14	Sulphur	
				Methomyl	
			24-Oct-14	Spirotetramat	
			23-Dec-14	Dichlorvos	
		Crimson Seedless	23-Sep-14	Sulphur	
			23-Sep-14	Methomyl	
			02-Oct-14	Sulphur	
			02-Oct-14	Methomyl	
			24-Oct-14	Spirotetramat	
			30-Dec-14	Dichlorvos	

Optenhorst	2014/2015	Dan Ben Hannah	15-Sep-14	Sulphur	Not disclosed
			15-Sep-14	Methomyl	
			22-Sep-14	Sulphur	
			22-Sep-14	Methomyl	
			01-Oct-14	Sulphur	
			01-Dec-14	Methomyl	
			05-Nov-14	Spirotetramat	
			30-Dec-14	Dichlorvos	
St Malo	2013/2014	Red Globe	23-Sep-13	Fenazaquin	Eriophyidae
			14-Oct-13	Fenazaquin	Eriophyidae
			30-Dec-13	Dichlorvos	Thripidae
		Regal	01-Oct-13	Fenazaquin	Eriophyidae
	2014/2015	Red Globe	05-Sep-14	Fenazaquin	Eriophyidae
			15-Sep-14	Fenazaquin	Eriophyidae
			06-Oct-14	Spinetoram	Thripidae
			16-Oct-14	Spinetoram	Thripidae
			27-Oct-14	Spinetoram	Thripidae
			05-Dec-14	Spinetoram	Thripidae
		Regal	20-Sep-14	Fenazaquin	Eriophyidae
			30-Sep-14	Fenazaquin	Eriophyidae
			13-Oct-14	Spinetoram	Thripidae
			23-Oct-14	Spinetoram	Thripidae
			05-Nov-14	Spinetoram	Thripidae

Appendix 3

Table 3.a: Location of traps baited with pheromone dispensers were placed. Highlighted GPS co-ordinates (bold text) indicates successful trapping of adult male *Holocacista capensis*.

Town	Cultivar	Moths Trapped/Day	GPS coordinates
		BERG RIVER	
Paarl	Alphonse	< 157	**33°43'04.1"S 18°57'49.2"E**
	Dan Ben Hannah	< 178	**33°43'08.2"S 18°57'50.9"E**
	Regal	< 166	**33°40'24.6"S 18°54'12.0"E**
	Crimson	< 129	**33°40'30.6"S 18°54'12.3"E**
Wellington	Red Globe	< 34	**33°35'48.2"S 18°58'33.4"E**
	Regal	< 65	**33°35'40.5"S 18°58'31.7"E**
Wolseley	Unknown (wine)	< 7	**33°24'47.7"S 19°14'06.7"E**
Riebeeck Kasteel	Unknown (wine)	< 116	**33°23'00.3"S 18°54'36.5"E**
	Alphonse	72	**33°23'22.6"S 18°53'37.3"E**
	Red Globe	< 62	**33°23'25.7"S 18°53'43.1"E**
Tulbagh	Unknown (wine)	< 137	**33°17'30.8"S 19°05'18.6"E**
	Unknown (wine)	< 21	**33°16'32.9"S 19°06'42.5"E**
Prince Alfred Hamlet	Shiraz	< 1	**33°14'53.6"S 19°19'54.6"E**
Porterville	Cabernet	< 10	**33°00'38.6"S 19°00'47.6"E**
Piketberg	Shiraz	< 8	**32°57'59.7"S 18°44'54.2"E**
	Chenin Blanc	120	**32°58'44.8"S 18°44'55.9"E**
		HEX RIVER	
De Doorns	Unknown (table grape)	< 1	**33°34'40.1"S 19°30'15.8"E**
	Unknown (table grape)	< 1	**33°34'17.6"S 19°30'22.1"E**
	La Rochelle	0	33°30'12.6"S 19°35'57.3"E
	Dauphine	0	33°30'12.1"S 19°35'59.6"E
	Unknown (table grape)	0	33°29'17.5"S 19°39'26.5"E
	Unknown (table grape)	0	33°26'12.3"S 19°40'51.1"E
	Unknown (table grape)	0	33°26'04.3"S 19°40'46.2"E
	Crimson	0	33°26'22.8"S 19°41'04.0"E
	Unknown (table grape)	0	33°26'38.9"S 19°41'22.3"E
Robertson	Chardonnay	< 1	**33°46'17.0"S 19°45'06.9"E**
McGregor	Unknown (wine)	< 2	**33°54'11.6"S 19°52'30.7"E**
Ashton	Unknown (wine)	< 1	**33°49'23.2"S 19°58'41.0"E**
Bonnievale	Unknown (wine)	0	33°51'46.1"S 19°59'12.8"E
	Unknown (wine)	0	33°57'46.1"S 20°09'02.4"E

OLIFANTSRIVIER			
Vredendal	Unknown (wine)	< 19	**31°41'21.2"S 18°30'20.3"E**
Klaver	Unknown (wine)	0	31°45'26.7"S 18°33'49.5"E
	Unknown (wine)	0	31°45'20.1"S 18°37'05.9"E
Trawal	Crimson	0	31°53'13.2"S 18°37'47.3"E
OTHER			
Stellenbosch	Unknown (wine)	< 175	**33°56'22.2"S 18°51'58.6"E**
	Unknown (wine)	< 149	**33°58'10.8"S 18°51'52.8"E**
Hermanus	Unknown (wine)	0	34°21'58.6"S 19°15'10.1"E
	Pinot Noir	< 1	**34°23'13.9"S 19°13'60.0"E**
	Syrah	0	34°19'30.7"S 19°21'49.7"E
Grabouw	Pinot Noir	0	34°10'19.2"S 19°04'38.2"E
	Unknown (wine)	0	34°11'12.8"S 19°01'14.3"E
	Savignon Blanc	0	34°12'11.9"S 19°00'16.2"E
Suurbraak	*R. tomentosa*	0	34°00'08.4"S 20°37'44.6"E
	R. tomentosa	0	33°59'53.4"S 20°40'43.2"E
	R. tomentosa	0	33°59'53.8"S 20°40'39.5"E

Appendix 4

Table 4.a: A record of the observed plant samples kept at the Compton Herbarium (Kirstenbosch).

LEAFMINING SPECIES/HABIT	PLANT SPECIES	YEAR	LOCALITY
Heliozelidae	*Rhoicissus digitata* (L. F) Gilg & Brandt	1963	Gonubie, East London, Eastern Cape (coastal bush)
		1969	Glentana Bay, George, Western Cape
		1972	Between Loerie Village and Loerie Forest, Port Elizabeth, Eastern Cape
		1973	Lake Sibayi, Natal (dune forest)
		1973	Mazeppa Bay, Maxambeni, Eastern Cape
		1979	Oslo Beach, Port Shepston, Natal (cocoons present)
		1979	Stutterheim, Eastern Cape
	Rhoicissus pauciflora (Burch. ex DC.) Planch.	1980	Baviaanskloof, Eastern Cape
	Rhoicissus rhomboidea (E. Mey ex Harv.) Planch.	1961	Mbabane Forest, Swaziland
	Rhoicissus revoilii Planch.	1958	Pigg's Peak, Swaziland
	Rhoicissus schlechteri Gilg & Brandt	1952	Hanglip, Naboomspruit, Waterberg, Limpopo
	Rhoicissus tridentata (L. F.) Wild & Drum.	No date	Groot Brakrivier, Western Cape
		1915	Mossel Bay, Western Cape
		1954	Potgietersrust, Mokopane, Northern Transvaal
		1961	Forbes Reef, Mbabane, Swaziland
		1967	Plettenberg Bay, Western Cape

Long-tailed mines	*Rhoicissus tomentosa* (Lam.) Wild & Drum.	1951	Swellendam, Western Cape
		1962	Knysna, Western Cape
	Rhoicissus tridentata (L. F.) Wild & Drum.	1923	Louis Trichardt, Limpopo (Eastern Valley, Blaauwberg, North Western Tranvaal)
		1950	24 miles East of Grahamstown, Eastern Cape
		1973	Blyde River Poort Dam, Sabie
	Cissus nymphaeifolia (Welw. ex Bak.) Planch.	1962	Ruacana, Namibia
Unknown (large red/yellow/brown blotch mines)	*Rhoicissus revoilii* Planch.	1972	Zambezia, Mozambique